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Organotolerance in the Natural Bacterial Assemblage in Surface Sediments of Charleston Harbor, San Diego Bay, and the Upper Delaware River System

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14. ABSTRACT

The addition of volatile organic compounds (VOCs) into the environment represents one stressor that may affect metabolism among some components of the bacterial assemblage in submerged sediments. Chronic exposure to VOCs and their rapid transport to submerged sediments may impact the structure of the assemblage by increasing the selective pressure for organotolerant strains. We developed an assay to differentiate the change in bacterial production in response to input of the VOC nephthalene. Bacterial production in surface water and sediments that chronically receive input of fresh petroleum or other volatile organics was less inhibited by nephthalene additions than was bacterial production from more pristine areas. The inherent difficulties involved in assessing current day input from historical contamination in estuaries have the potential to make this a valuable tool in environmental forensics. This assay was used to evaluate the bacterial assemblage in surface sediments of three coastal estuaries chosen because they all involve differentiating historical petroleum releases by the Navy from current industrial inputs to estuarine sediments.

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EXECUTIVE SUMMARY

The addition of volatile organic compounds (VOCs) into the environment represents one stressor that may affect metabolism among some components of the bacterial assemblage in submerged sediments. Chronic exposure to VOCs and their rapid transport to submerged sediments may impact the structure of the natural bacterial assemblage by increasing the selective pressure for organotolerant strains. We developed an assay to differentiate the change in bacterial production in response to input of the VOC, naphthalene. Bacterial production in surface water and sediments that chronically received input of fresh petroleum or other volatile organics was less inhibited by naphthalene additions than those from more pristine areas. The inherent difficulties involved in assessing current day input from historical contamination in estuaries have the potential to make this a valuable tool in environmental forensics. This assay was used to evaluate the bacterial assemblage in surface sediments of three coastal estuaries: Charleston Harbor, San Diego Bay, and the upper Delaware River system. These three sites were chosen because they all involve differentiating historical petroleum releases by the Navy from current industrial inputs to estuarine sediments. Samples from most stations exhibited some decrease in production with increase in the amount of naphthalene added to the heterotrophic production assay. At a couple of stations, naphthalene actually stimulated bacterial production, though this only happened with two of the 64 measurements. The two stations were adjacent to known outfalls of volatile organics; Charleston Station 4 near a paper mill outfall, and Philadelphia Station 9 near another industrial outfall. Although we do not know the exact time scales involved from exposure to adaptation, in this survey we were able to identify two sediment stations that consistently harbored bacterial assemblages that were organotolerant. This report represents one line of evidence that VOCs are being released at such a rate from these two industrial outfalls that the natural bacterial assemblage in adjacent sediments is frequently impacted. Although microorganisms are not considered a receptor in estuarine ecosystems, this assay provides an understanding of microbial community structure that could help assess sublethal effects in contaminated sediments and act as an early-warning system for effects on higher organisms.

ORGANOTOLERANCE IN THE NATURAL BACTERIAL ASSEMBLAGE IN SURFACE SEDIMENTS OF CHARLESTON HARBOR, SAN DIEGO BAY, AND THE UPPER DELAWARE RIVER SYSTEM

INTRODUCTION

Each gram of estuarine sediment contains approximately 10^9 individual bacterial cells, representing 10^3 to 10^4 cells of separate genetic groups (phylotypes) (Torsvik et al. 1990). Collectively, this is referred to as the natural bacterial assemblage. Its composition is a result of numerous environmental factors and stressors, such as the presence of volatile organics, which can disrupt the bacterial cell membrane and cause cell damage or death (see review by Beney and Gervais 2001). Bacteria can compensate for the presence of these compounds by decreasing the fluidity of their cell membrane, making it less susceptible to disruption by organic compounds. This is known as organotolerance. While membrane fluidity can be altered by the bacterium, sudden changes in the volatile organic concentration may cause a temporary reduction in the metabolic rate of cells that are not adapted to elevated organic levels. Conversely, a bacterial assemblage that is chronically exposed to volatile organics is likely to be less metabolically affected by abrupt change in ambient organic concentration.

The component of the bacterial community that can metabolize organic compounds to bacterial biomass and carbon dioxide is called the heterotrophic assemblage. The metabolic rate of the heterotrophic assemblage, also called secondary or bacterial production, is commonly measured using the leucine incorporation assay (Kirchman et al. 1985, Smith and Azam 1992, Kirchman 1993). The rate of bacterial incorporation of a radiolabeled amino acid (^{3}H-leucine) into cellular proteins correlates with the assemblage growth rate as bacteria synthesize proteins when growing and dividing. The amount of amino acid incorporated can then be converted to the bacterial biomass produced in terms of carbon metabolism (for instance, µg C produced per gram dry weight sediment per day) (Simon and Azam 1989). In addition to its widespread use in marine microbial ecology, bacterial production has been successfully used to evaluate hydrocarbon biodegradation strategies in groundwater (Holm et al. 1992, Jensen 1989, Boyd et al. 2001, Boyd et al. 2002, Montgomery et al. 2002) and wastewater inputs into groundwater (Harvey and George 1987, Harvey et al. 1984).

The addition of volatile organic compounds (VOCs) into the environment represents one stressor that may affect metabolism among some components of the bacterial assemblage in submerged sediments (Godoy et al. 1998). In estuaries, these compounds are often associated with surface water release of fresh petroleum fuels, atmospheric input, storm water runoff, and occasionally intrusion of contaminated groundwater into the submerged sediments. Inputs to the surface water can interact with surface sediments via resuspension, deposition of organics on particles, or through reworking of sediments by benthic macrofauna, which increases contact with the water column. Because of the abiotic and biotic losses of volatile organics from the system, VOC-impacted sediments might be expected to be closer to the source of the input than those sediments impacted by less volatile polycyclic aromatic hydrocarbons (PAH), like phenanthrene and fluoranthene. Chronic exposure to VOCs (Hayes et al. 1999) and their rapid transport to submerged sediments may impact the structure of the natural bacterial assemblage by increasing the selective pressure for organotolerant strains.

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One of the most commonly occurring VOCs in estuarine systems is naphthalene as it is associated with petroleum fuels, combustion activities, and many industrial processes. Despite its ubiquity in anthropogenically influenced systems (Kastner et al. 1994), naphthalene is transient as it is metabolized by many types of natural bacteria and is more water soluble and volatile than many other petroleum compounds. This two-ringed hydrocarbon can rapidly transfer out of an estuarine system into the atmosphere (Gustafson and Dickhut 1997), so it is more likely to reflect a current petroleum source than a more weathered product input (Arzayus et al. 2001). Naphthalene has been reported to decrease bacterial diversity (Nyman 1999) and inhibit bacterial metabolism, even among strains that metabolize higher molecular weight PAH (Bouchez et al. 1995, Lantz et al. 1997). When coupled with the leucine incorporation assay, this inhibition may allow for the identification of sediments that are chronically subjected to inputs of volatile organics.

We developed an assay to differentiate the change in bacterial production in response to input of the VOC, naphthalene. It is expected that bacterial production in surface water and sediments that chronically receive input of fresh petroleum or other volatile organics will be less inhibited by naphthalene additions than those from more pristine areas. The inherent difficulties involved in assessing current day input from historical contamination in estuaries have the potential to make this a valuable tool in environmental forensics. This naphthalene inhibition assay was used to evaluate the bacterial assemblage in surface sediments of three coastal estuaries: Charleston Harbor, San Diego Bay, and the upper Delaware River system. These three sites were chosen because they all involve differentiating historical petroleum releases by the Navy from current industrial inputs to estuarine sediments. This is one part of a comprehensive evaluation by NRL of PAH biodegradation and transport in coastal ecosystems.

METHODS AND MATERIAL

Study Site and Collection Methods

Charleston Harbor

This survey involved seasonal sampling of 22 stations through the Charleston Harbor and the three major rivers that are part of the watershed: the Ashley, the Wando, and the Cooper (Fig. 1). This study site includes the former Charleston Navy Yard (CNY), which is situated along the Cooper River about one mile upriver from the Charleston Harbor. Ten of the stations were within the area adjacent to the former CNY on the Cooper River (Stations 6A, 6B, 6C, 6D, 6E, 9, and 13) and Shipyard Creek (Stations 10, 11, and 13). Two stations (16 and 17) were near the mouth of the Ashley River. Two stations (A2 and A3) were in the Wando River. Three stations (14, 15, and A1) were in the Charleston Harbor. Sample size was dependent on the type of sample analysis to be conducted. Water samples were collected using a standard shipboard CTD rosette that held three 10-L Niskin collection bottles. Surface water was collected 1 m below the estuary surface. Bottom water was collected from 1 m above the sediment-water interface. Water samples were taken in acid-cleaned 500 mL amber glass bottles with Teflon®-lined closures. Water samples used for biological analysis were transferred to onboard laboratory facilities for processing within minutes of collection. Surface sediment (top 10 in.) was collected using a Shipek benthic grab. Sediment samples for PAH and biological analyses were transferred to 50 mL centrifuge tubes and immediately subsampled for mineralization and production assays.

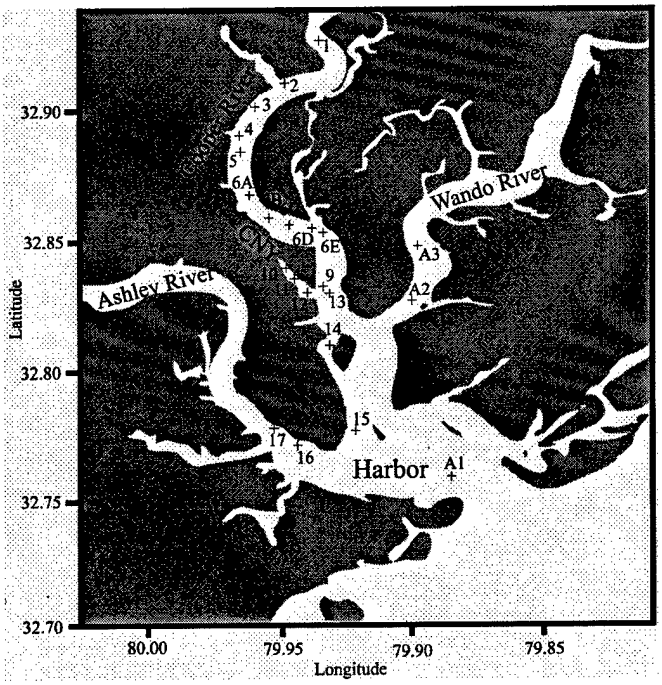

Fig. 1 — Charleston Harbor sampling stations

San Diego Bay

Surface sediments were sampled in the San Diego Bay aboard the R/V *Ecos* using a Petite Ponar benthic grab. Samples were collected from six stations (TS01, TS02, TS03, PC07, PC08, and PC08) within Paleta Creek, which is near Naval Station San Diego (Table 1). In addition, two stations were collected from outside this study site near Shelter Island (SDB) and Coronado Cayes (Pristine). Surface water samples were collected with a 2.5 L Nansen-type bottle.

Table 1 — GPS Coordinates for the November 1999 Sampling of Surface Sediments in San Diego Bay around Paleta Creek, Shelter Island, and Coronado Cayes

Location	Station Code	Longitude	Latitude
Shelter Island	SDB	117.2276000	32.7160500
Coronado Cayes	Pristine	117.1360833	32.6378000
Paleta Creek	TS01	117.1160056	32.6741583
Paleta Creek	TS02	117.1159105	32.6736903
Paleta Creek	TS03	117.1159013	32.6732012
Paleta Creek	PC07	117.1174583	32.6721536
Paleta Creek	PC08	117.1177001	32.6724453
Paleta Creek	PC09	117.1179399	32.6727709

Upper Delaware River System

This study was conducted in a tidally influenced freshwater region of the Delaware River near Philadelphia, Pennsylvania (USA) (Fig. 2, from Boyd et al. 1999). A series of stations was established in the industrialized region of the Delaware and Schuylkill Rivers, where environmental impacts have been well documented (Weisberg and Burton 1993, Maxted et al. 1997, Steyermark et al. 1999). Samples were also taken within the Philadelphia Naval Complex Reserve Basin (RB; Fig. 3, from Boyd et al. 1999) during research cruises in December 1998 and May 1999. Sediment samples were collected using a Petite Ponar within the RB from an *Avon* inflatable and with a Smith-Mack grab from the R/V *Cape Henlopen* in the Delaware and Schuylkill Rivers. Water samples were collected with a 2.5 L Nansen-type bottle in the RB and a 30 L Niskin bottle in the Delaware and Schuylkill Rivers.

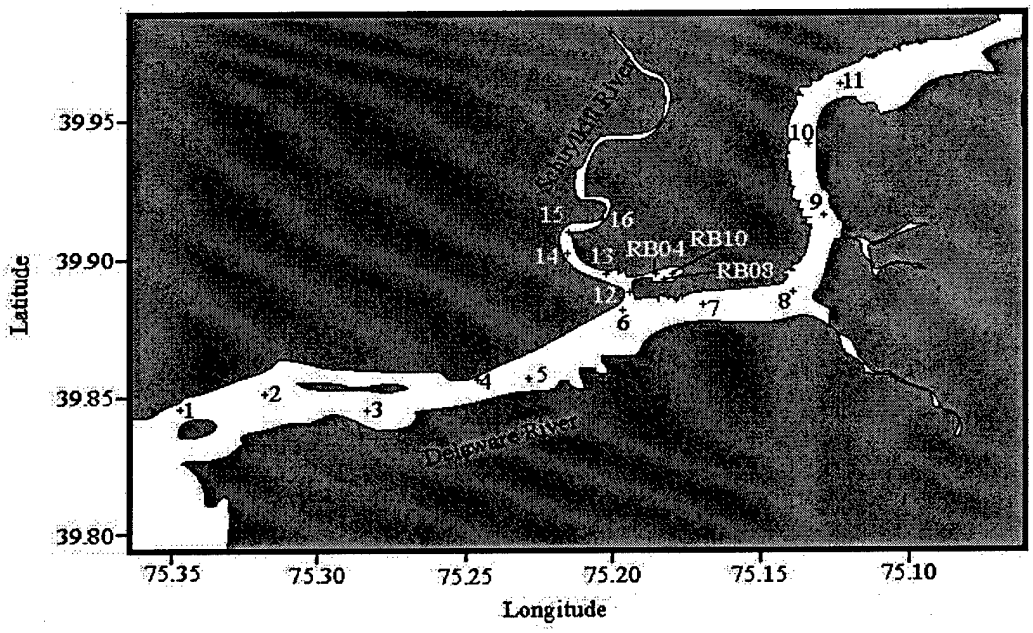

Fig. 2 — Delaware and Schuylkill sampling stations

Bacterial Production Assay

A 50-µL sample of wet sediment was subsampled from each station and added to 2.0-mL centrifuge tubes (three experimental and one killed control) that were precharged with [3H-4,5]-L-leucine (154 mCi mmol-1, final concn. 20 nM) and killed with 57 mL of 100% trichloroacetic acid (Sigma). The sediment was extracted from the benthic grab sample and added to the 2.0 mL tube using a 1 mL polypropylene syringe with the end cut off. One mL of 0.22 µm (nom. pore dia.) filtered bottom water (collected less than 1 m above bottom) was then added to each tube to form a sediment slurry. Samples were incubated for 1 to 2 hours at *in situ* temperatures and subsequently processed by the method of Smith and Azam (1992). A constant isotope dilution factor of 2.0 was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids (Burdige and Martens 1990) and saturation experiment estimates (Tuominen 1995). Triplicate 1-mL syringed samples of wet sediment were dried at 50 °C and used to convert production values to dry weight. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989).

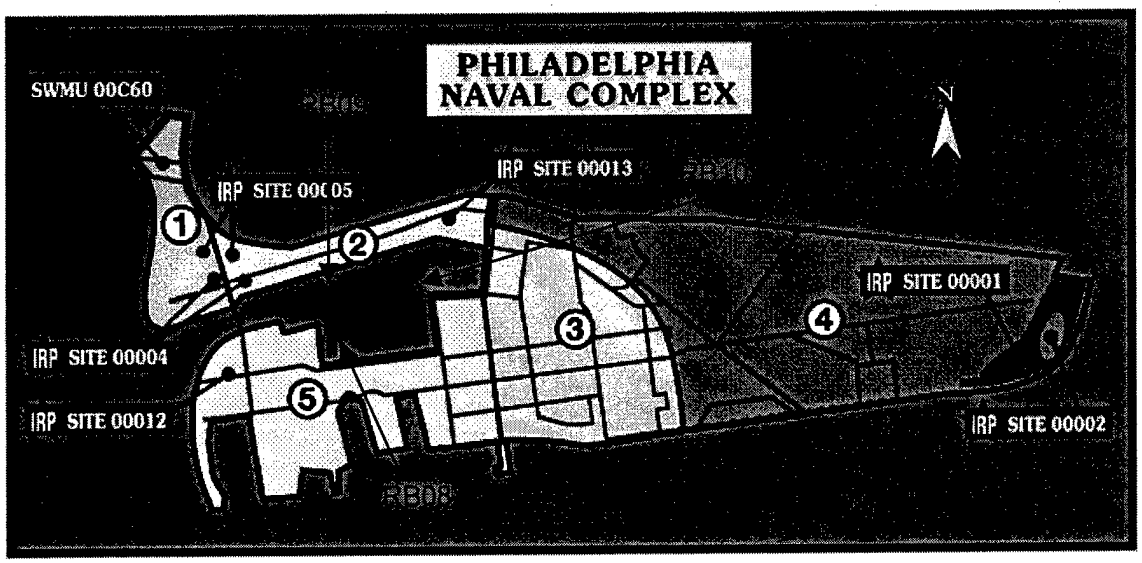

Fig. 3 — Philadelphia Reserve Basin sampling stations

Naphthalene Inhibition Assay

Organotolerance of the natural bacterial assemblage to naphthalene was measured by addition of 0, 5, 15 or 25 μg of naphthalene dissolved in 5 μL of methanol to 0.50 μL of wet sediment samples with subsequent processing for production (Montgomery et al. 1999). All treatments and controls received the same addition of methanol (5 μL) though preliminary experiments with parallel incubations with no methanol added showed that production was not statistically affected. Controls in the results presented have methanol added to the incubations.

Statistics

Average values for production were used in regressions with the amount of naphthalene added to the leucine incorporation assay. The formula and the r^2 value were calculated using Excel.

RESULTS

At 58 sediment stations and 8 water column stations, the effect of naphthalene additions was measured on heterotrophic bacterial production (Table 2). The leucine incorporation method was used to measure bacterial production of a natural assemblage from the rate of incorporation of ^{3}H-leucine incorporated into bacterial proteins. The measure is an average for the assemblage and as such is insensitive to competing affects such as stimulation of one component of the assemblage simultaneous with the inhibition of another component of the assemblage. An increase in one component can offset the decrease in productivity of another and be measured as "no effect." Heterotrophic bacterial assemblages are likely to respond in one of three ways to the addition of naphthalene:

1. decrease rates of production as the cell membrane of organosensitive cells is disrupted and the cells are damaged or lysed;

2. maintain rates of production as organotolerant cells are unaffected by the presence of naphthalene; or
3. increase rates of production and organotolerant cells that can metabolize naphthalene in response to the addition of this relatively labile carbon source.

Table 2 — Summary of All Water and Sediment Samples Assayed for Naphthalene Inhibition

Date	Sediment	Surface Water	Bottom Water
Charleston (39 total)			
Aug-98	1, 2, 3, 4, 10, 12, A2	1, 2, 3, 4, 6A, 6E	6B, 6E
Dec-98	1, 2, 3, 4, 5, 6E, 17, A2		
Apr-99	1, 4, 5, 6A, 12, 17, A1, A2		
Jun-99	1, 4, 5, 6C, 12, 17, A2, 20		
San Diego Bay (8 total)			
Nov-99	TS01, TS02, TS03, Pristine, PC07, PC08, PC09, SDB		
Upper Delaware River (11 total)			
Dec-98	3, 9, 10		
May-99	RB04, RB08, RB10, 3, 6, 10, 12, 16		

Charleston Harbor

Water and surface sediments were collected from the Charleston Harbor Estuary over four research cruises in August and December 1999 and April and June 2000. At least seven stations were sampled during each cruise and an additional eight stations were sampled for surface or bottom water in August 1999. In addition to historical input from Naval operations at the former Charleston Navy Yard, possible sources of VOCs to the surface sediments in the study are numerous industries along the river and harbor waterways including a petroleum storage facility and paper mill, as well as storm water and surface runoff (Van Dolah et al. 1990).

Linear regression of the station averages described the effect of naphthalene on inhibiting bacterial production ($r^2 > 0.8$) with four surface water samples (August Stations 1, 2, 4, 6A; Figs. 4 through 7) and two from bottom water (August Stations 6B, 6E; Figs. 8 and 9), but did not describe the effect in surface water at August Stations 3 or 6E ($r^2 < 0.8$; Figs. 10 and 11). In these latter two samples, no effect was observed ($r^2 = 0.152$ and 0.324, respectively). Surface water at Station 3 was collected 2 h after high tide so it was possible that it was influenced by the outfall effluent from Station 4 moving upriver with the tide. Tidal influence and the high salinity stratification between surface and bottom water in this section of the Cooper River (Van Dolah et al. 1990) complicate interpretation of these findings.

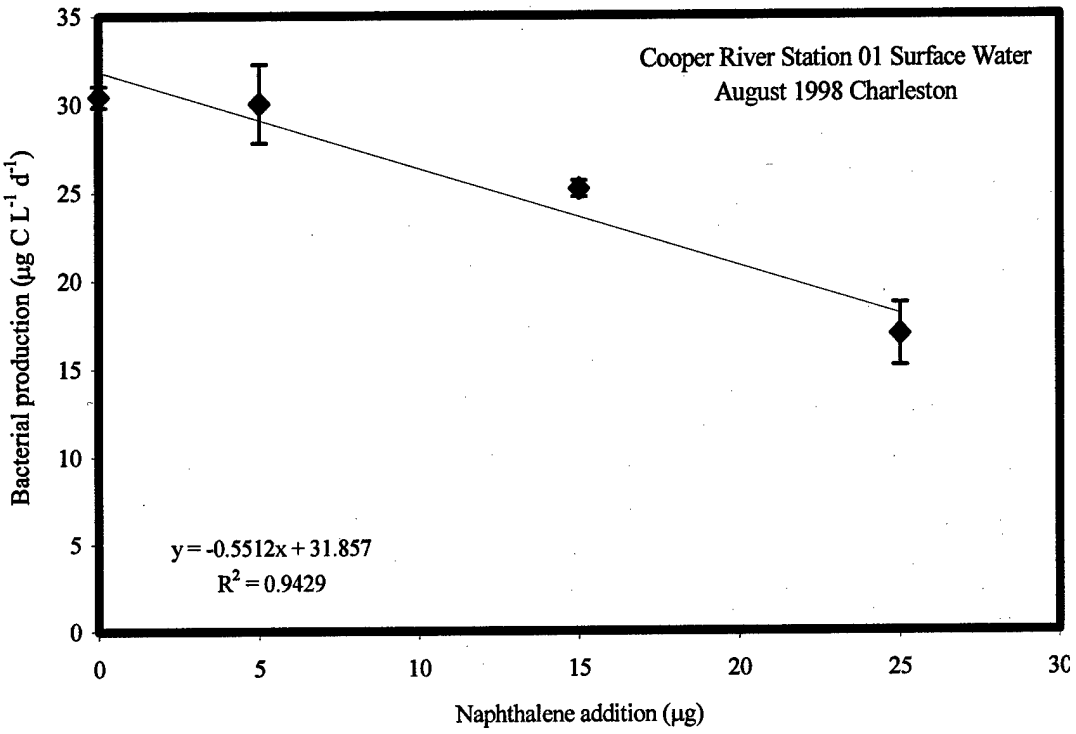

Fig. 4 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the surface water of the Cooper River station 01 during August 1998

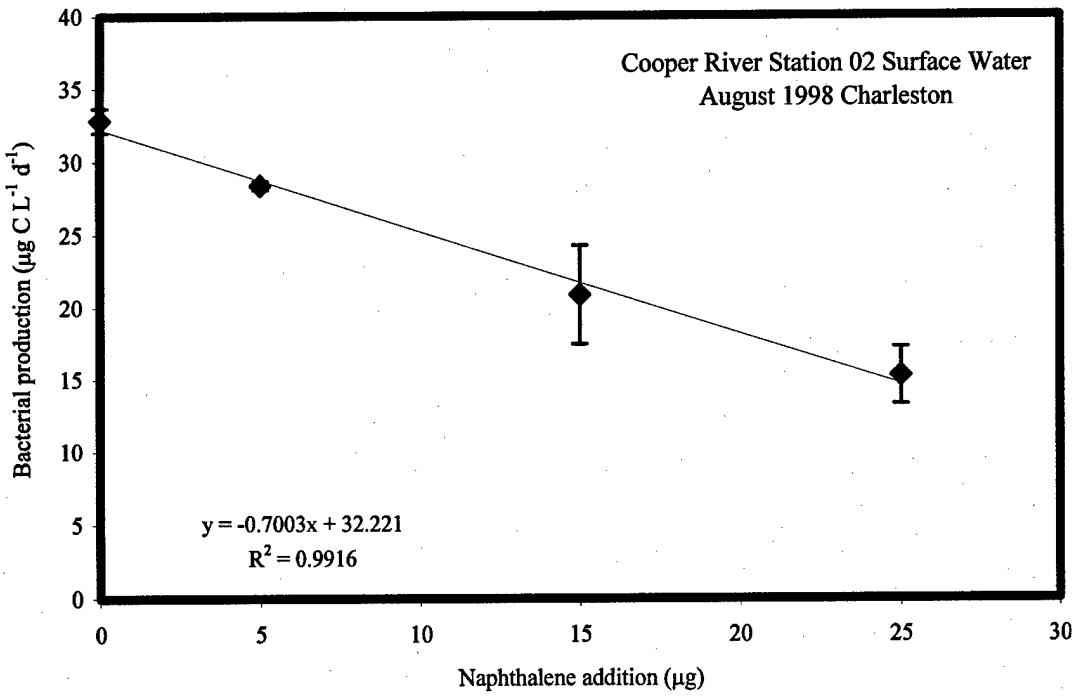

Fig. 5 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the surface water of the Cooper River station 02 during August 1998

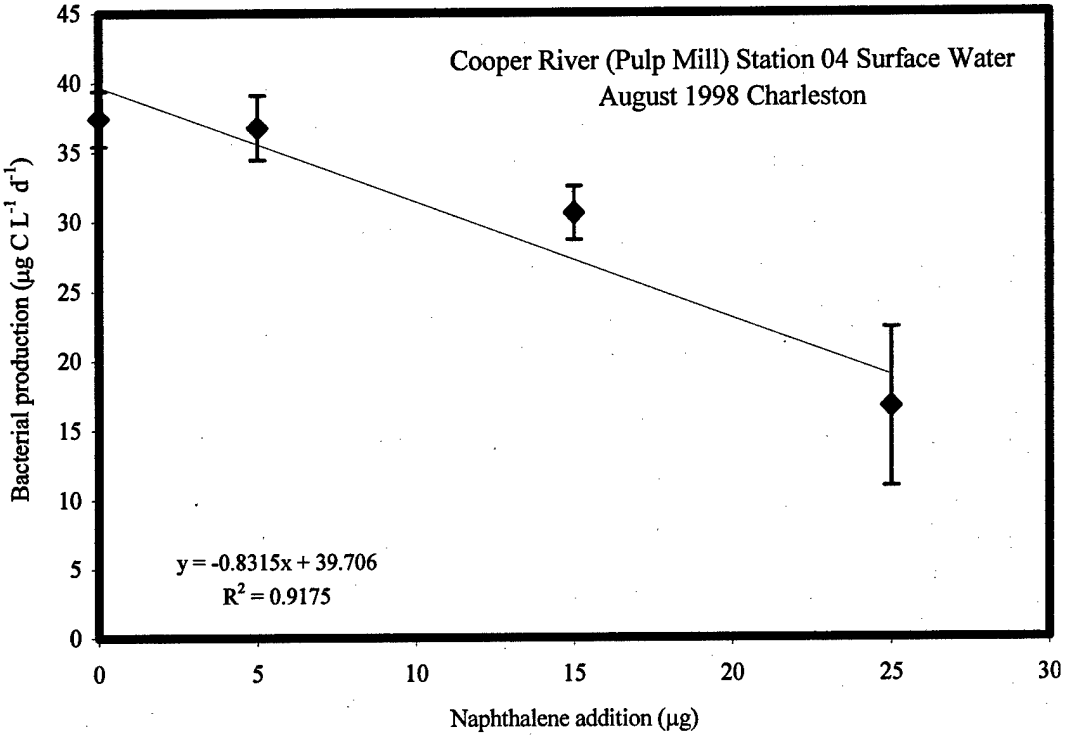

Fig. 6 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the surface water of the Cooper River station 04 during August 1998

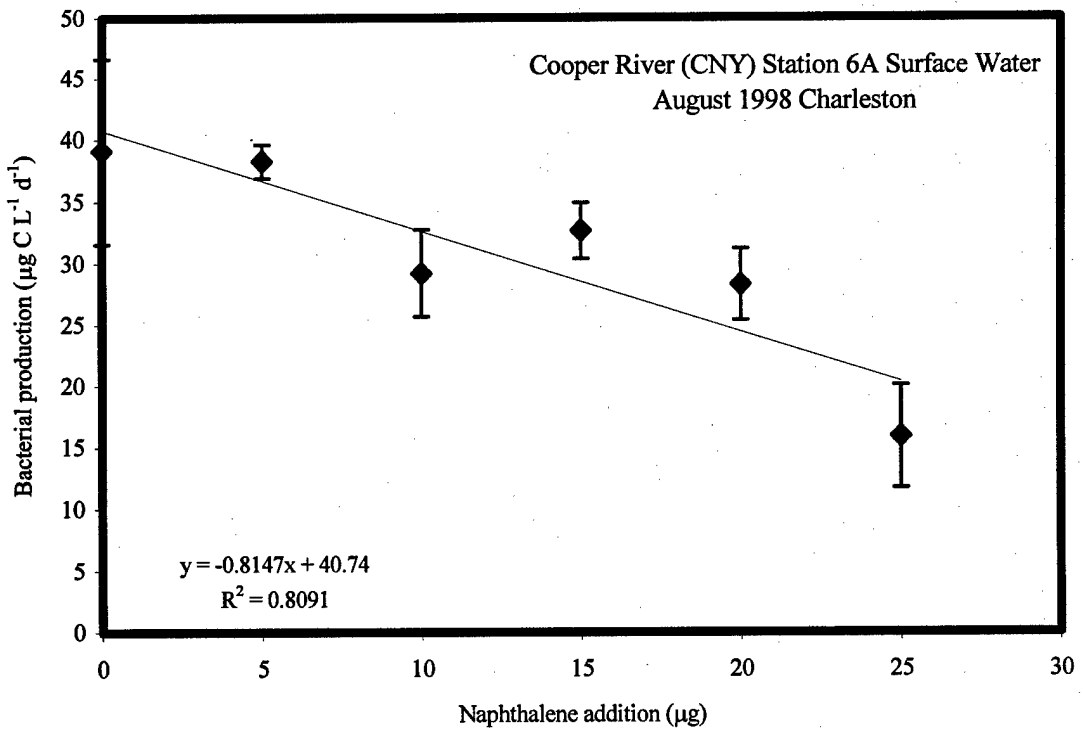

Fig. 7 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the surface water of the Cooper River station 6A during August 1998

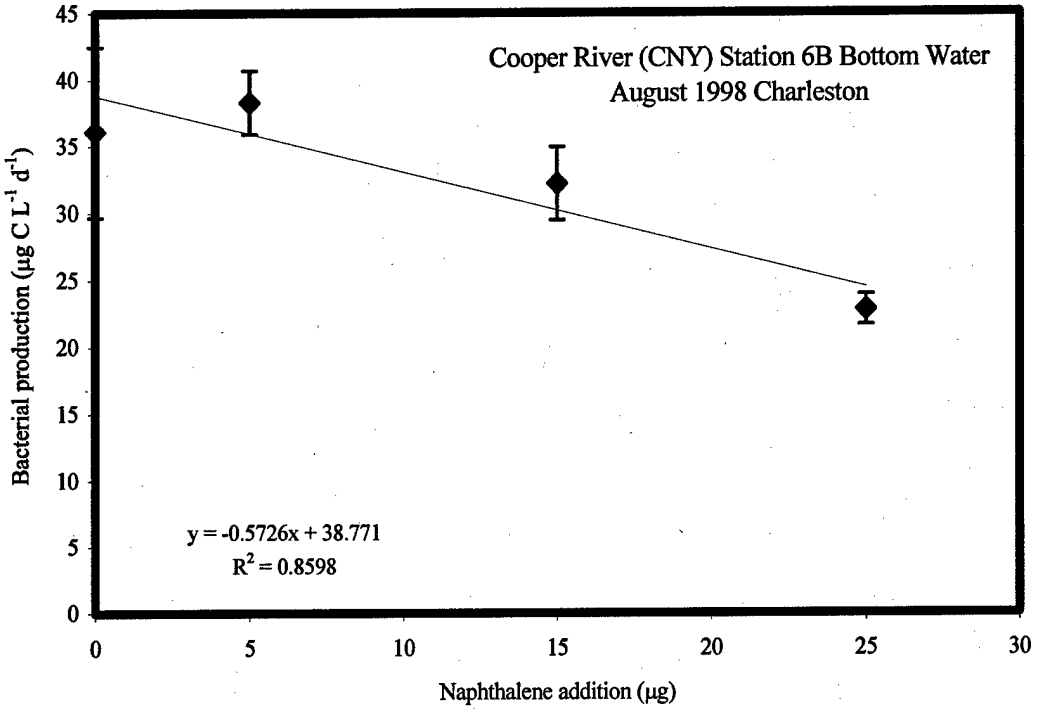

Fig. 8 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the surface water of the Cooper River station 6B during August 1998

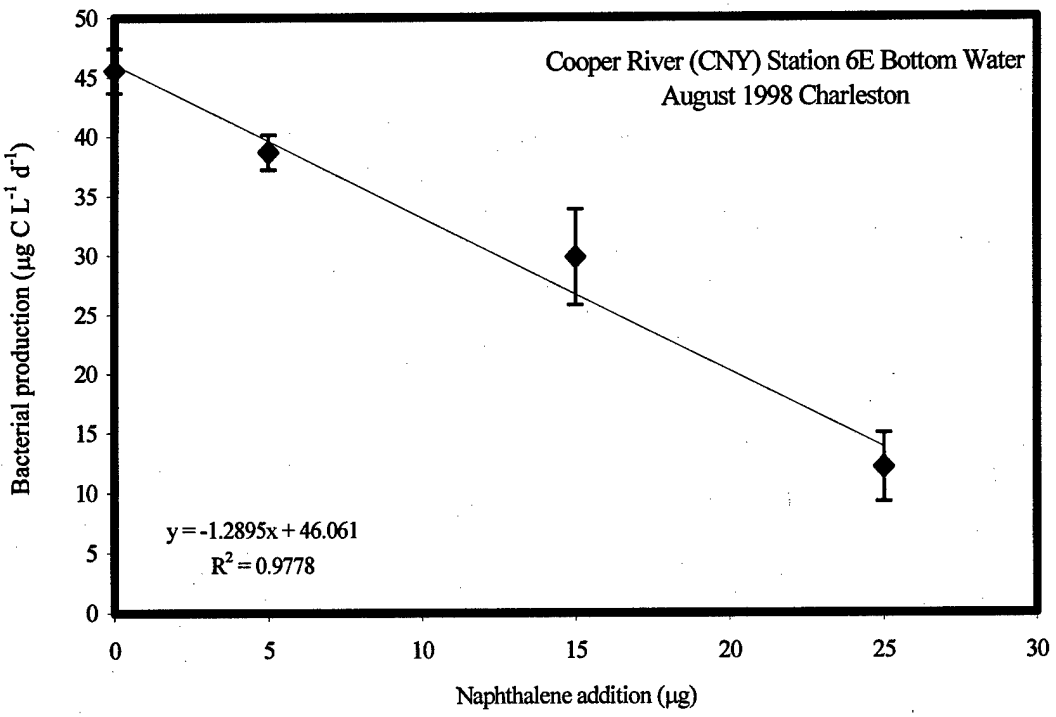

Fig. 9 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the bottom water of the Cooper River station 6E during August 1998

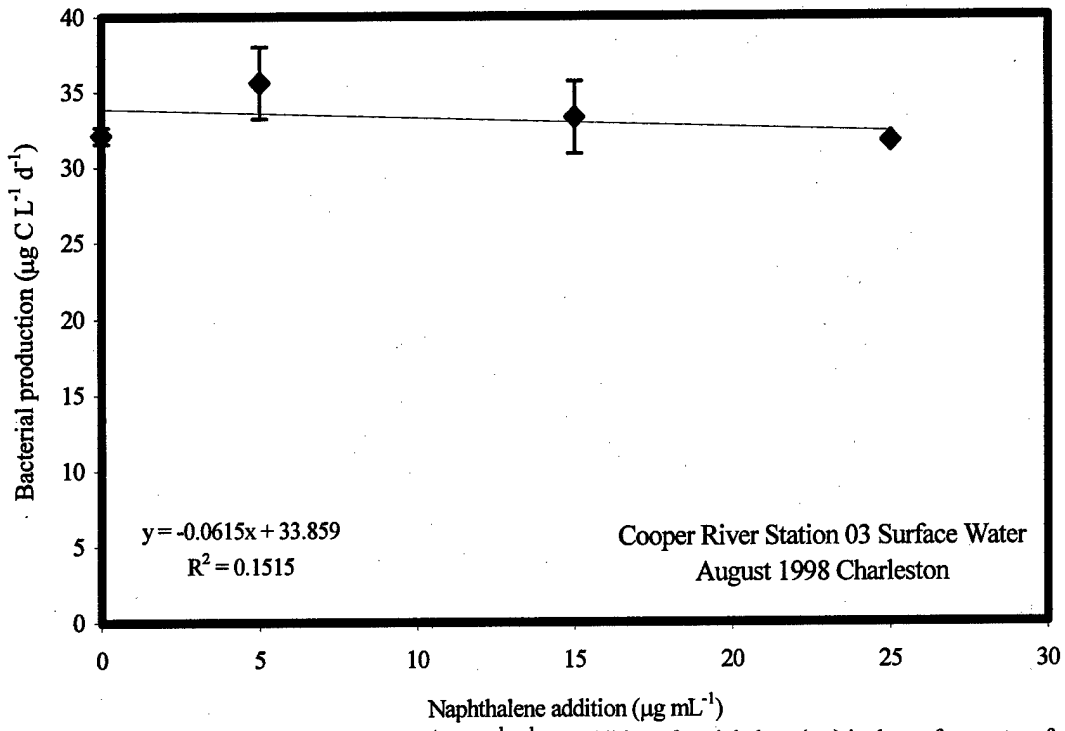

Fig. 10 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the surface water of the Cooper River station 03 during August 1998

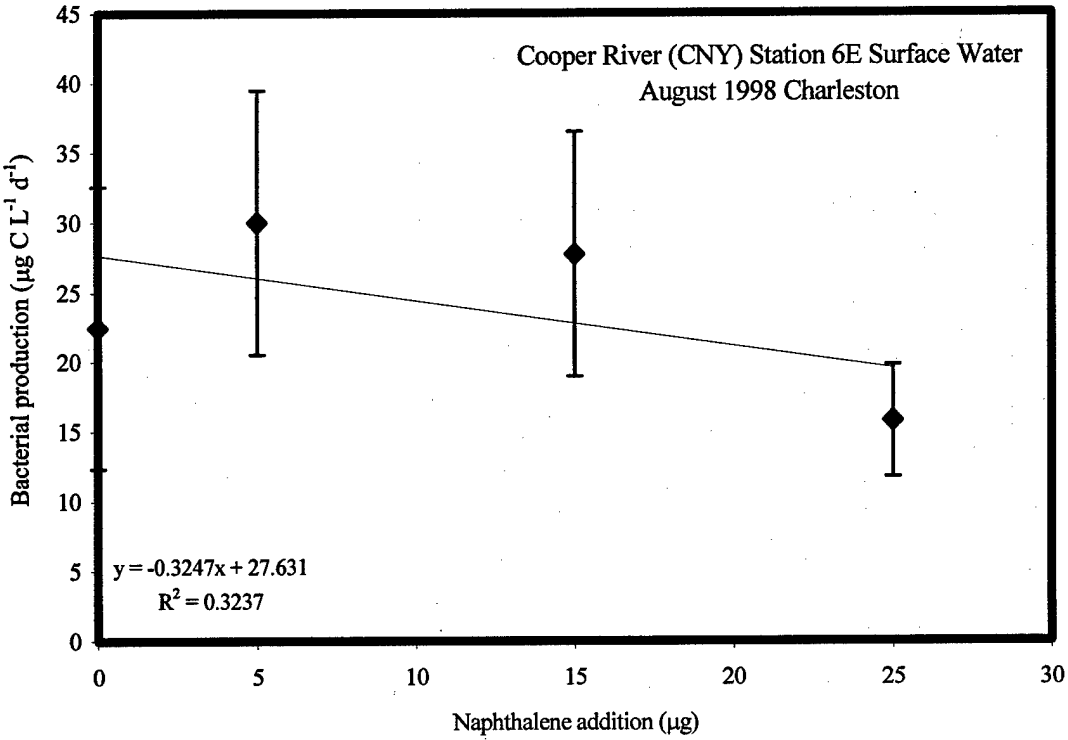

Fig. 11 — Inhibition of bacterial production (µg L^{-1} d^{-1}) by addition of naphthalene (µg) in the surface water of the Cooper River station 6E during August 1998

Linear regressions described the inhibitory effect of naphthalene on bacterial production in surface sediments in 15 out of the 34 samples taken during the four cruises ($r^2 > 0.8$). Production was linearly inhibited on more than one sampling, for three stations outside of the Cooper River, including the Wando River station A2 (August, December, April; Figs. 12 through 14), the Ashley River Bridge station 17 by the municipal marina (December, April, June; Figs. 15 through 17), and at station 12 near the mouth of Shipyard Creek (August, April, June; Figs. 18 through 20). Sediments from station 10 nearest the headwaters in Shipyard Creek were inhibited during one cruise (August; Fig. 21). Three stations in the Cooper, upriver of the Charleston Navy Yard were inhibited by naphthalene at least once, including stations 1 (April; Fig. 22), 2 (August; Fig. 23), and 5 (December; Fig. 24). Several stations in the Cooper, upriver of the CNY, were unaffected by increasing amounts of naphthalene including stations 2 (December; Fig. 25), 4 (April; Fig. 26) and 5 (April; Fig. 27) with one station nearest the paper mill showing some stimulation by the naphthalene (station 4, August, $r^2 = 0.638$; Fig. 28).

A third type of effect was observed with the addition of naphthalene to surface sediment samples. In nine of the observations, low concentrations of naphthalene (5 µg) inhibited production relative to the control but higher concentrations did not show more inhibition than the control. As a result, the linear regression did not accurately describe the relationship between naphthalene addition and reduced production ($r^2 < 0.8$), but these samples did show a decrease in production relative to the control. Many of these samples were from the Cooper River, including Stations 1 (August, December, June; Figs. 29 through 31), 3 (December; Fig. 32), 4, and 5 (both June; Figs. 33 and 34). One station was from the Ashley River, 20 (June; Fig. 35), one from Charleston Harbor, A1 (April; Fig. 36) and two were from the Cooper adjacent to the CNY, 6A (April; Fig. 37) and 6C (June; Fig. 38).

The remaining four samples were not described by the regression and high discrepancy between sample replicates within a treatment made it difficult to determine whether there was a complex relationship with naphthalene addition, problem with the assay, or sediment sample heterogeneity higher than those from the rest of the survey. These samples were from the Wando River station A2 (June; Fig. 39), the CNY station 6E (December; Fig. 40), near the paper mill Station 4 (December; Fig. 41) and upriver in the Cooper Station 3 (August; Fig. 42). The latter two stations showed inhibition of production at low naphthalene additions but much less inhibition at higher additions. It is possible that low naphthalene concentrations inhibited production by a component of the assemblage but were not enough to stimulate production by the naphthalene-metabolizing assemblage. At higher naphthalene concentrations, production by the naphthalene-metabolizing assemblage countered reductions in the organosensitive assemblage.

San Diego Bay

In November 1999, the surface sediment at eight stations in San Diego Bay were sampled including Poleta Creek (TS01, TS02, TS03, PC07, PC08, PC09), and two stations away from the creek at Shelter Island (SDB) and Coronado Cayes (Pristine). Possible sources of VOCs to the surface sediments include storm water and surface water runoff into Poleta Creek, intrusion of contaminated groundwater, and Navy ship activity (Katz 1998). Linear regressions described the inhibitory effect of naphthalene on production at five of the eight stations ($R2 > 0.85$) including stations Pristine, TS01, TS02, TS03, and PC07 (Figs. 43 through 47). Naphthalene inhibition was demonstrated at the remaining three stations (PC08, PC09, and SDB; Figs. 48 through 50), but the effect was not linear with a decrease in production with the 5-µg addition and little further inhibition at higher concentrations. In addition, production of the unamended control was much lower at PC09 (13 µg C g^{-1} d^{-1}) than at the other stations (range: 43 to 126 µg C g^{-1} d^{-1}).

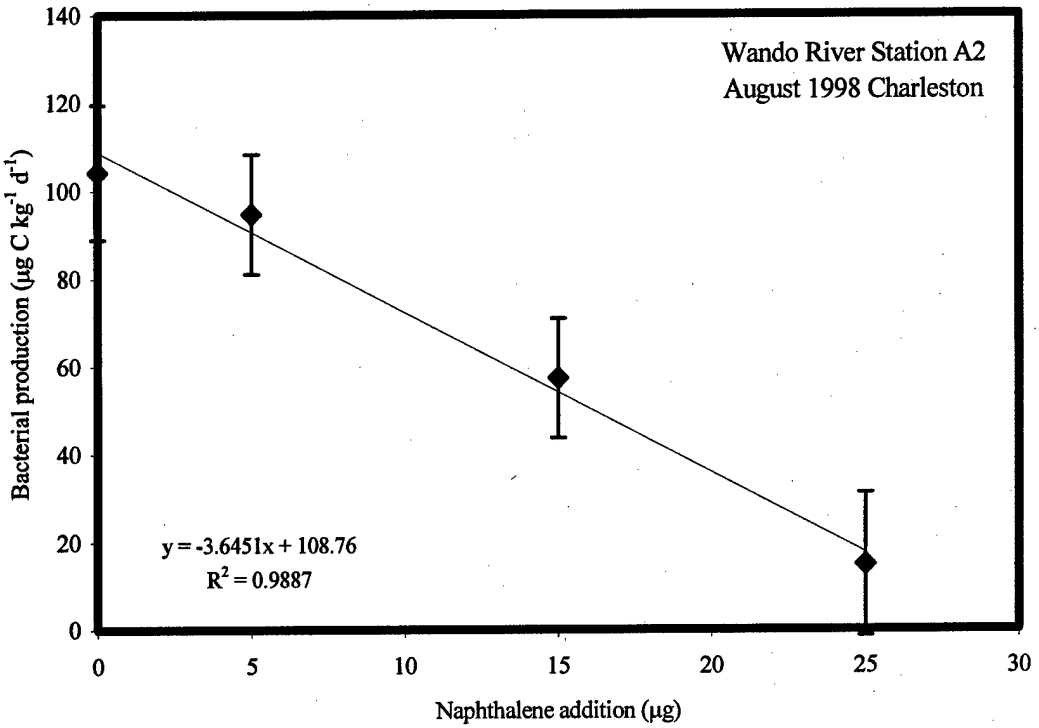

Fig. 12 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Wando River station A2 during August 1998

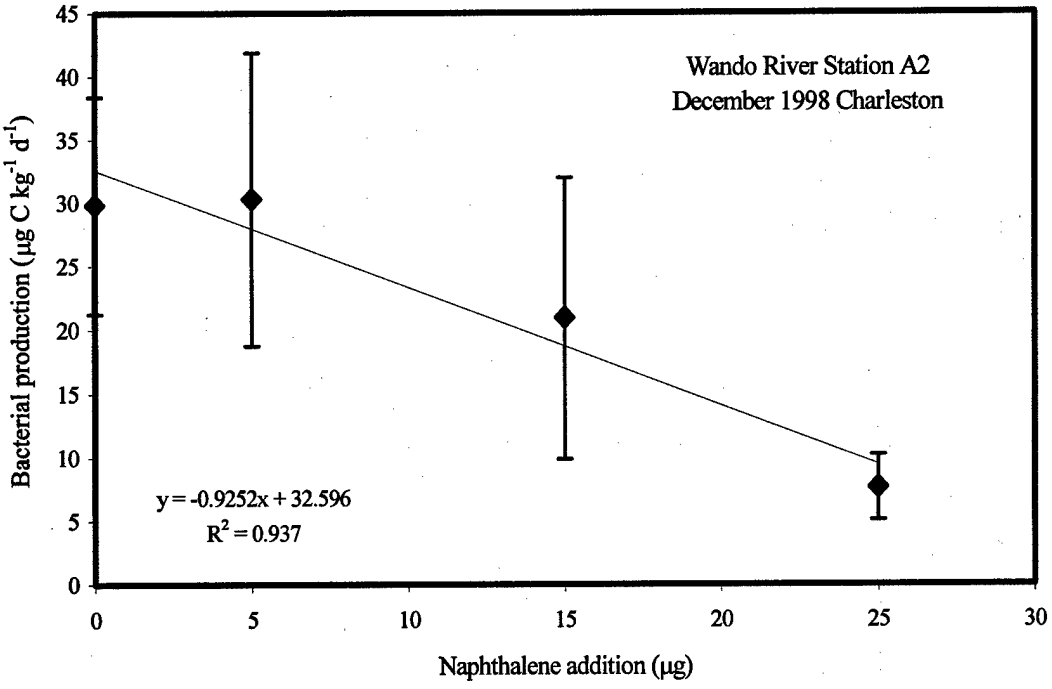

Fig. 13 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Wando River station A2 during December 1998

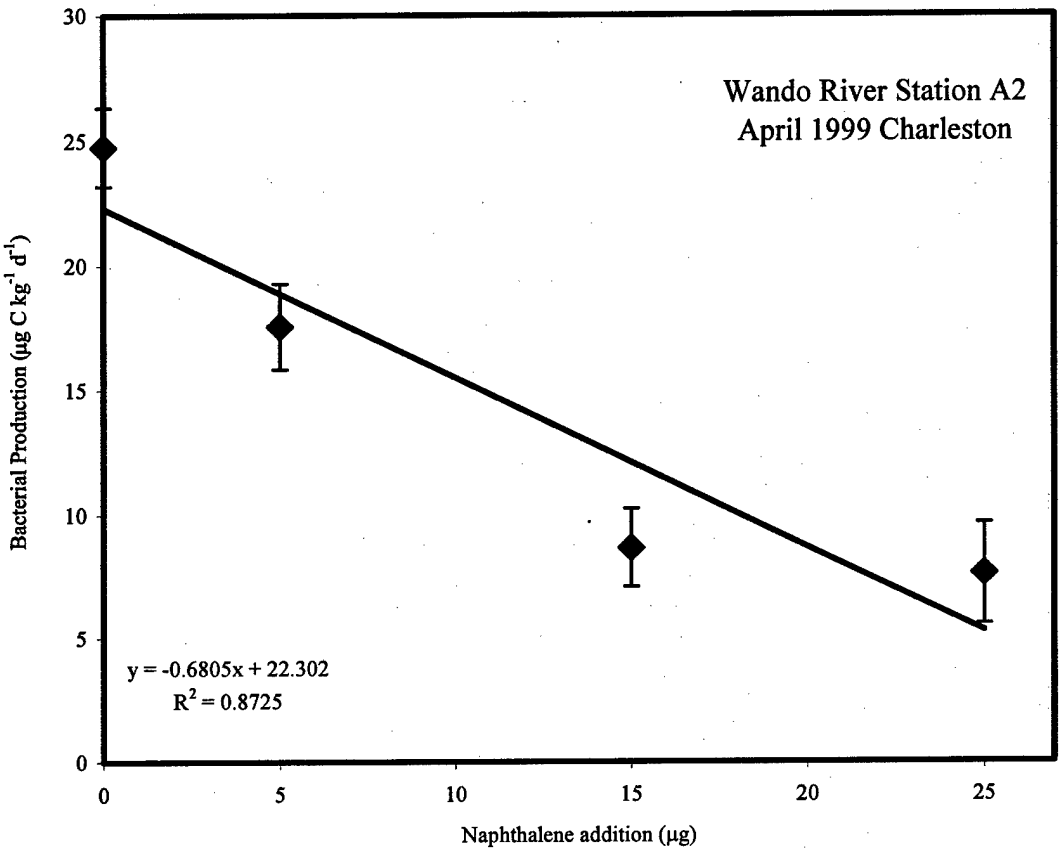

Fig. 14 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Wando River station A2 during April 1999

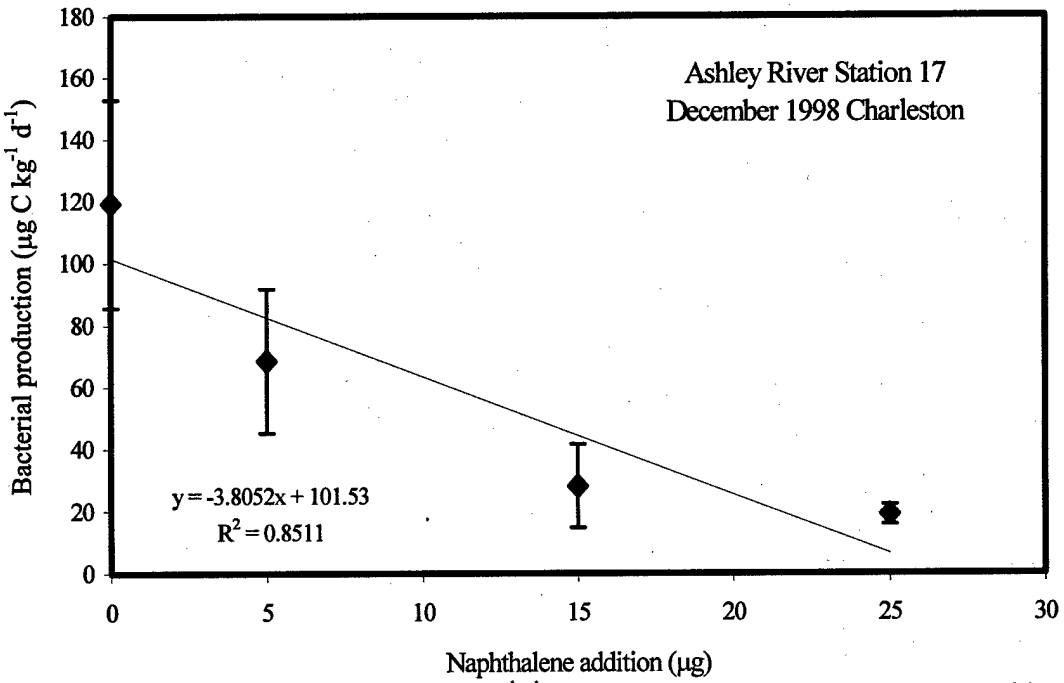

Fig. 15 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Ashley River station 17 during December 1998

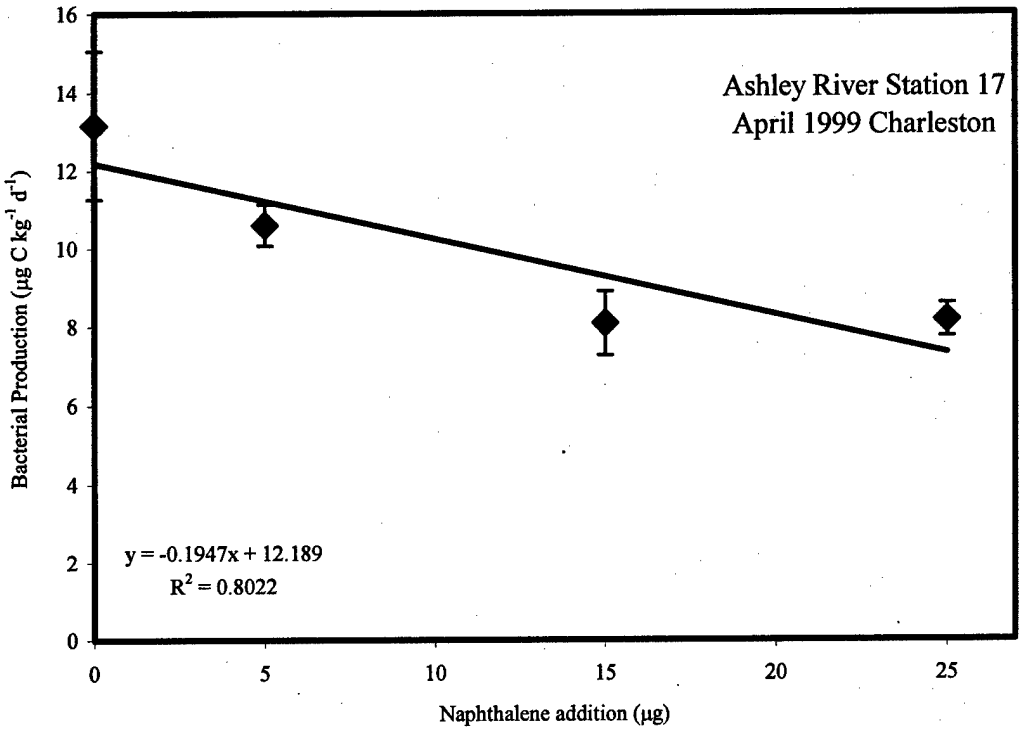

Fig. 16 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Ashley River station 17 during April 1999

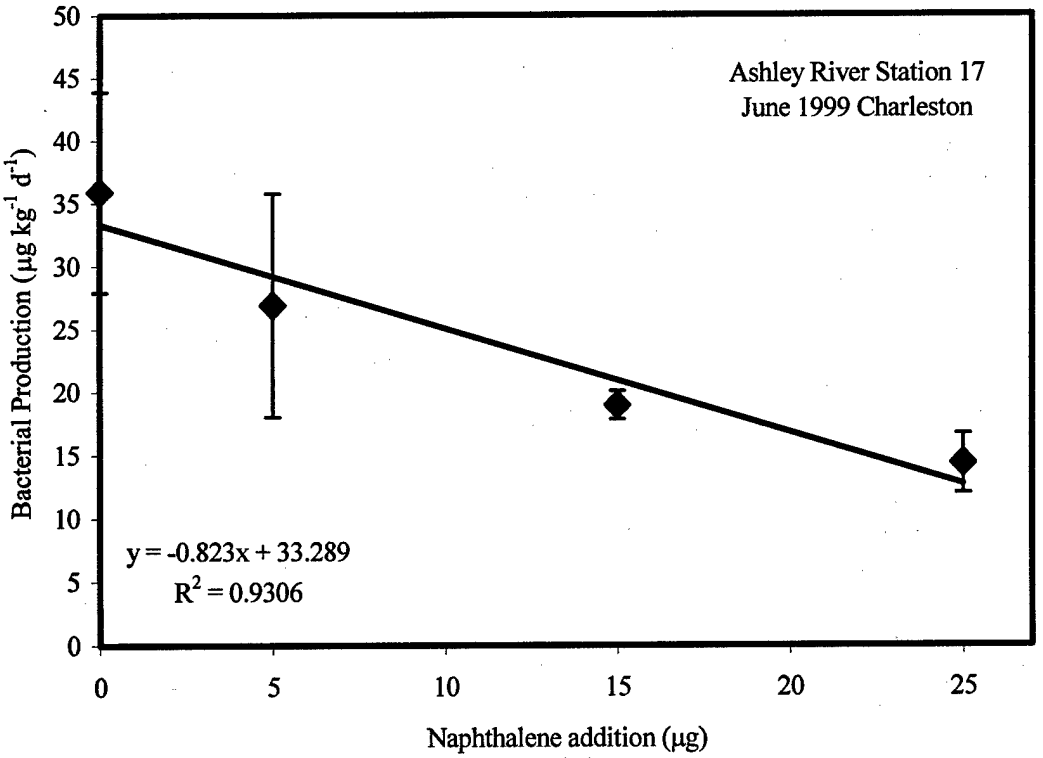

Fig. 17 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Ashley River station 17 during June 1999

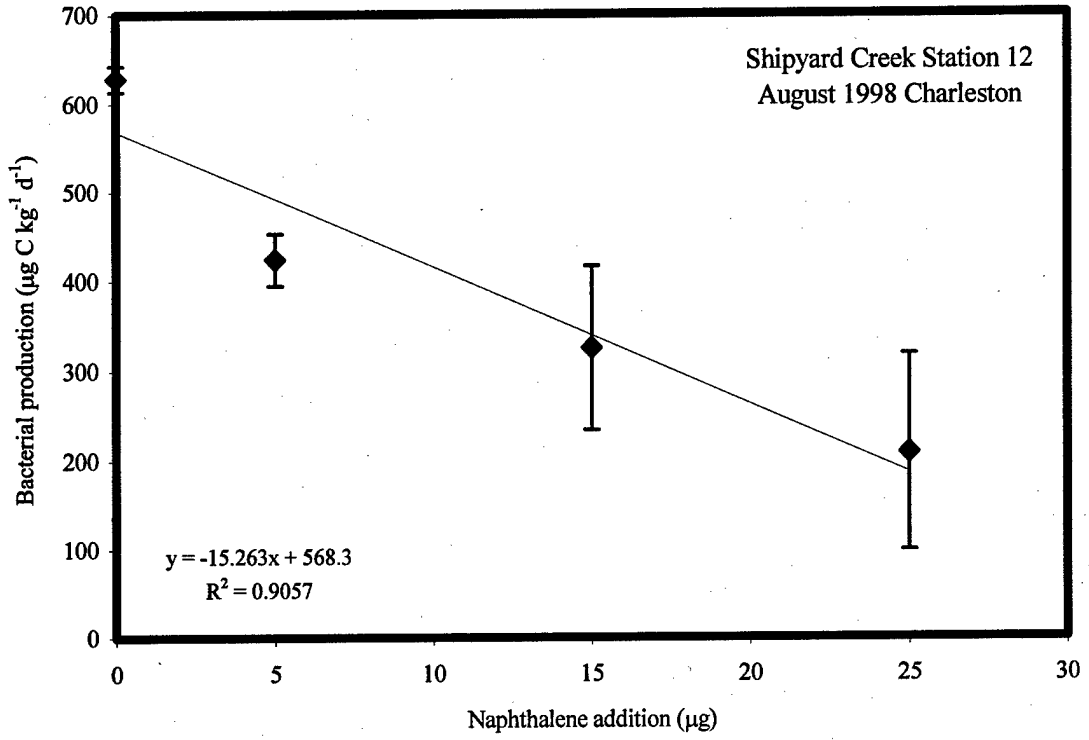

Fig. 18 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of Shipyard Creek station 12 during August 1998

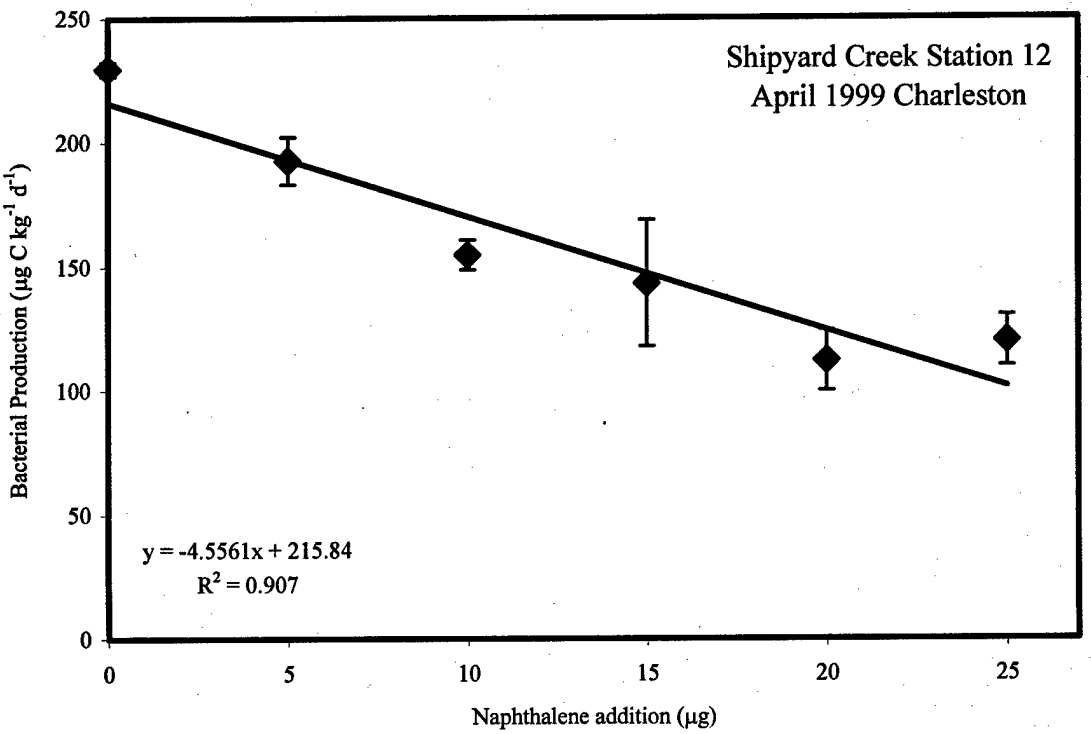

Fig. 19 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of Shipyard Creek station 12 during April 1999

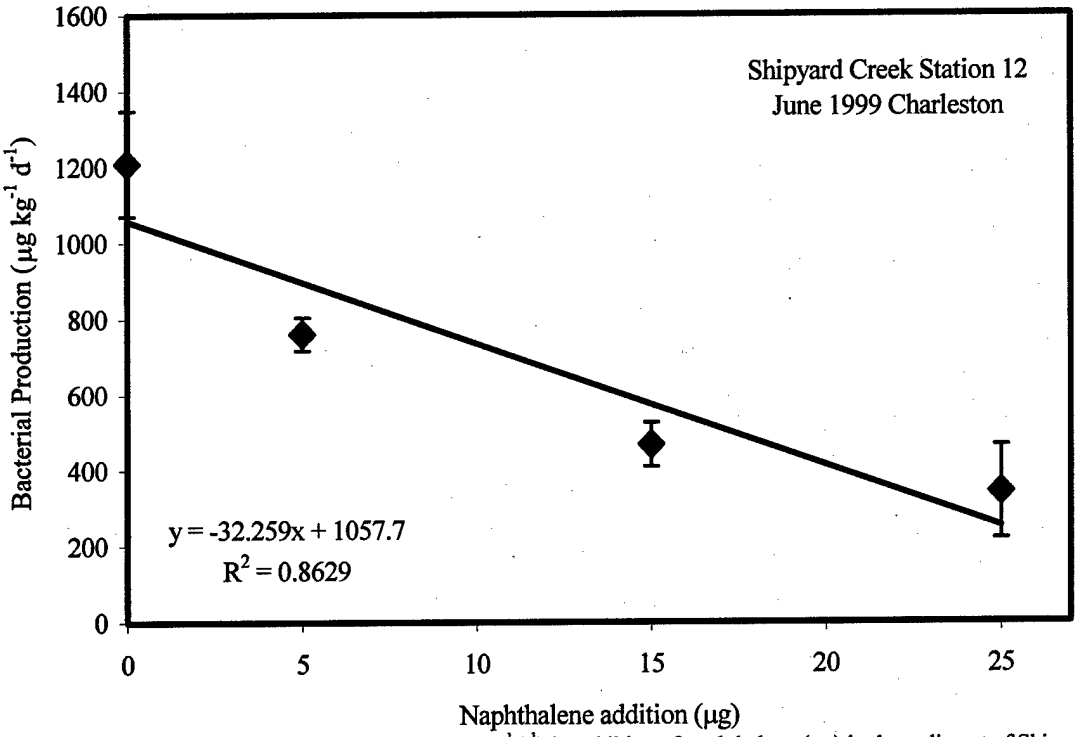

Fig. 20 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of Shipyard Creek station 12 during June 1999

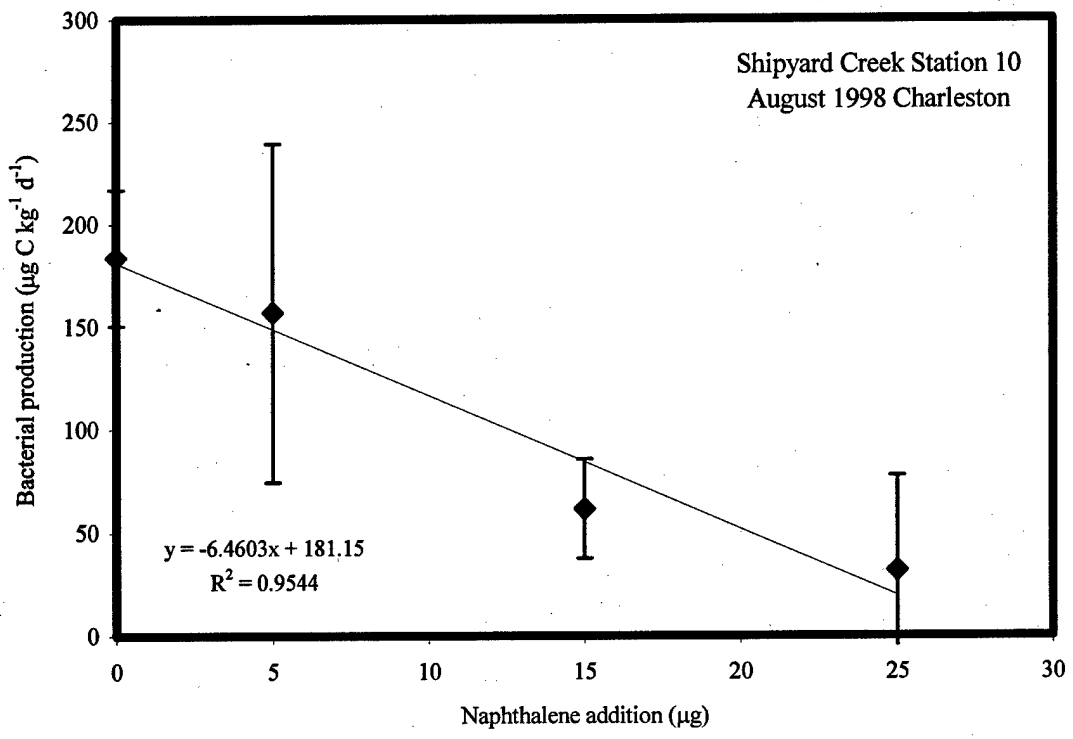

Fig. 21 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of Shipyard Creek station 10 during August 1998

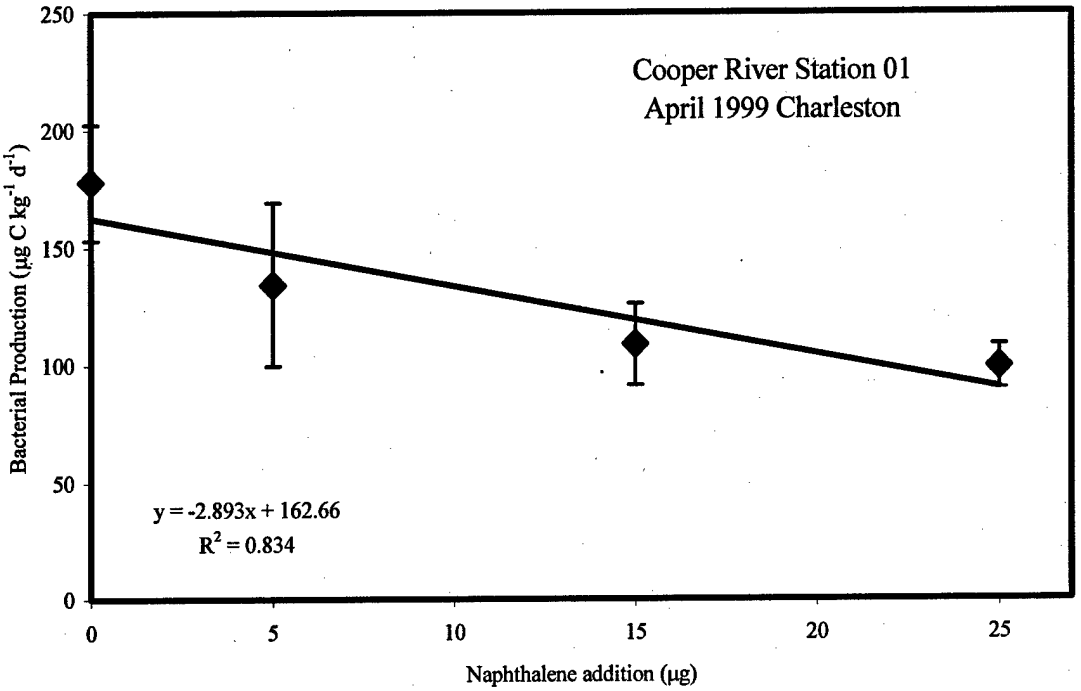

Fig. 22 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 01 during April 1999

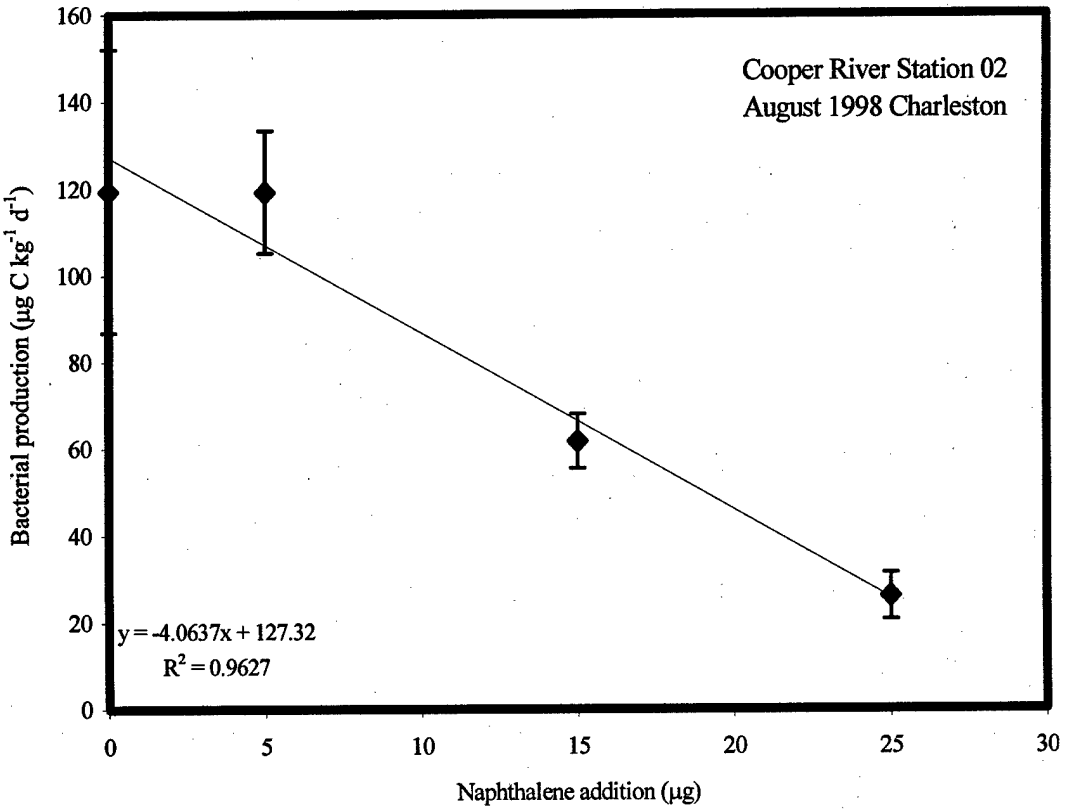

Fig. 23 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 02 during August 1998

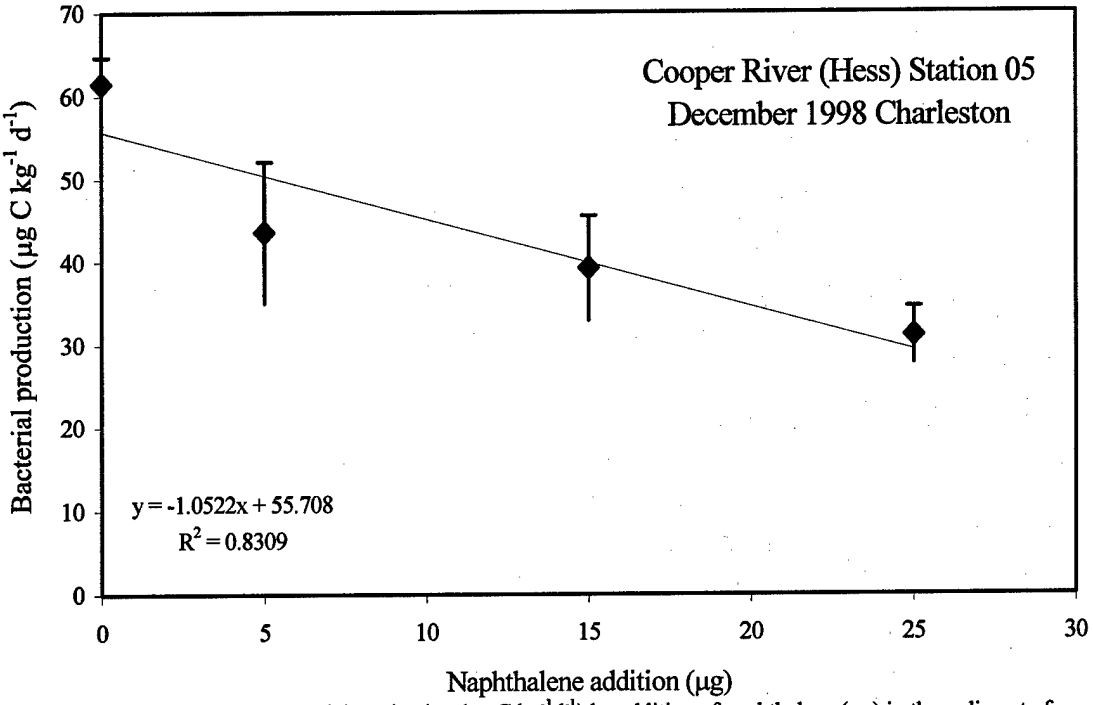

Fig. 24 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 05 during December 1998

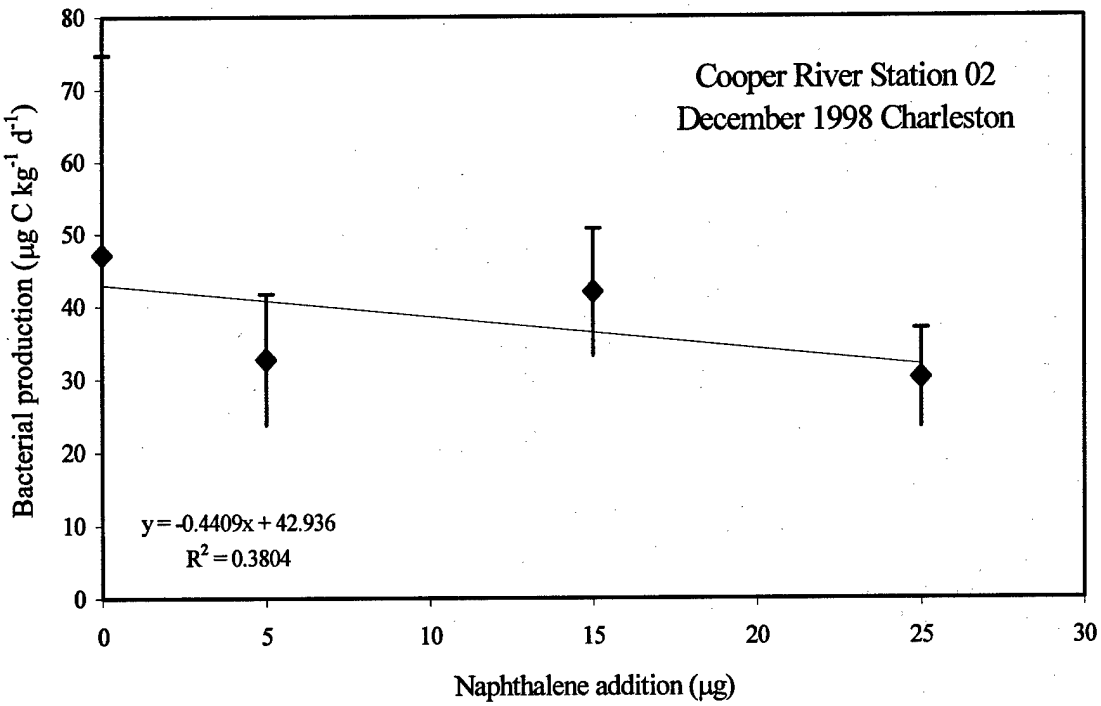

Fig. 25 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 02 during December 1998

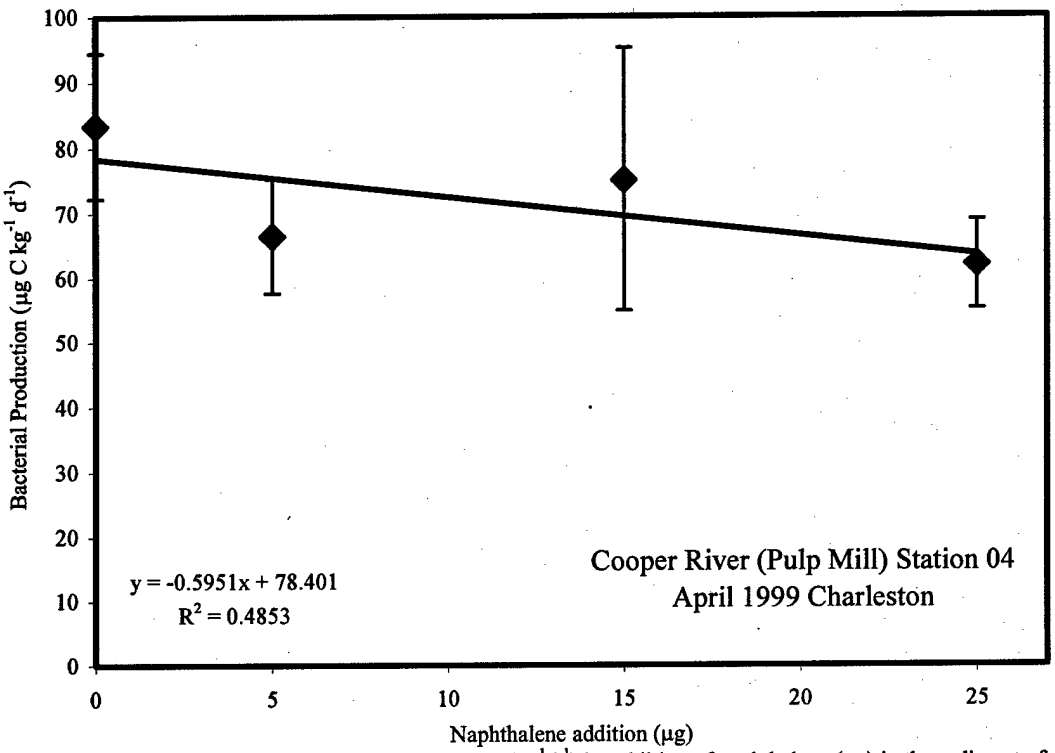

Fig. 26 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 04 during April 1999

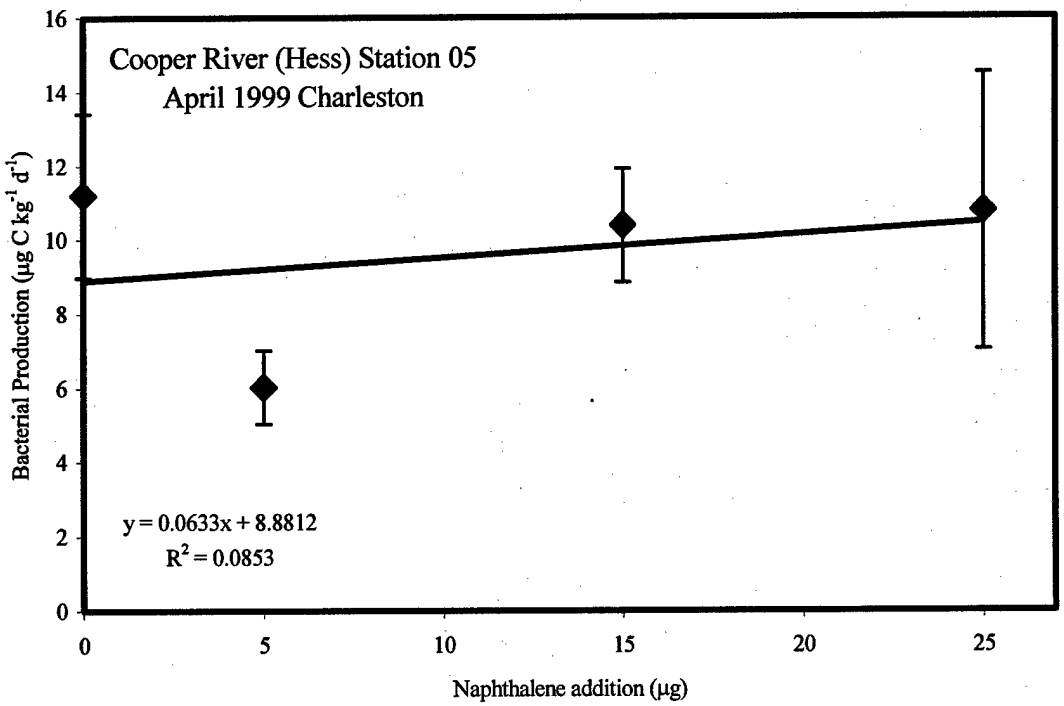

Fig. 27 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 05 during April 1999

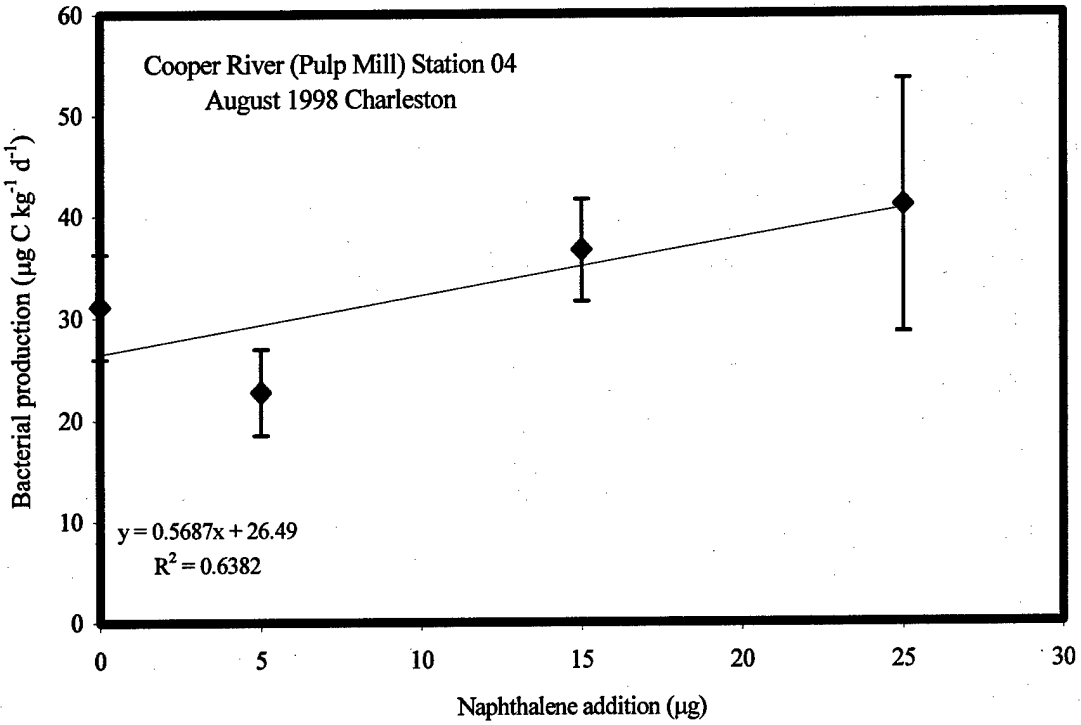

Fig. 28— Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 04 during August 1998

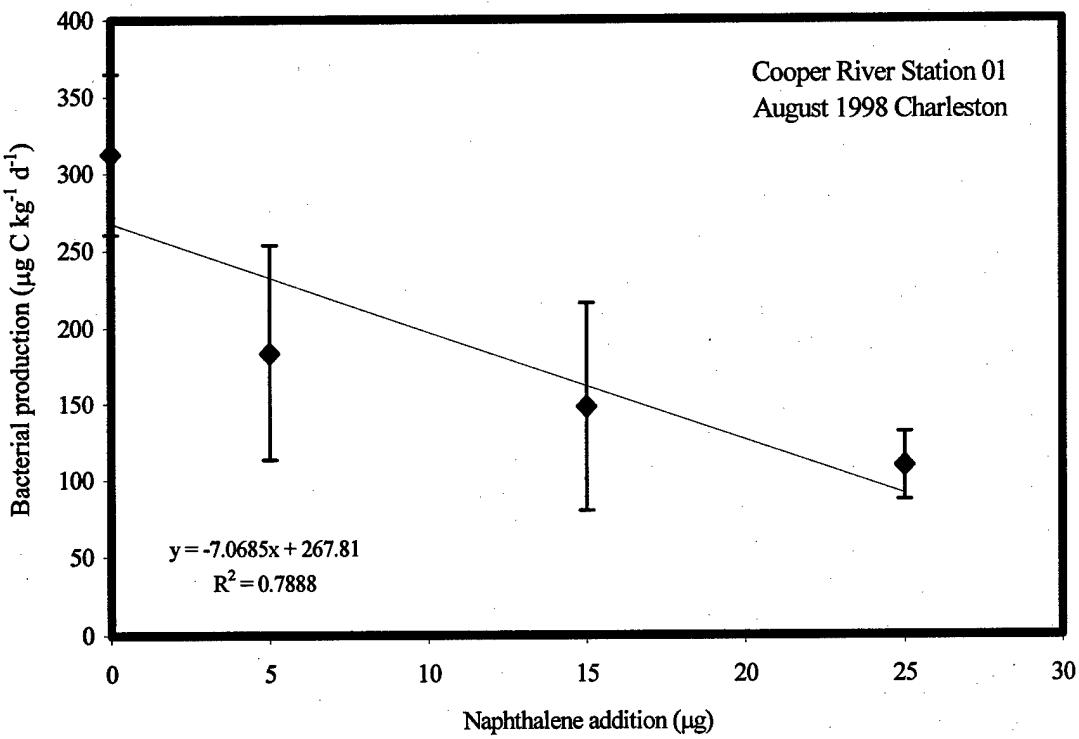

Fig. 29— Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 01 during August 1998

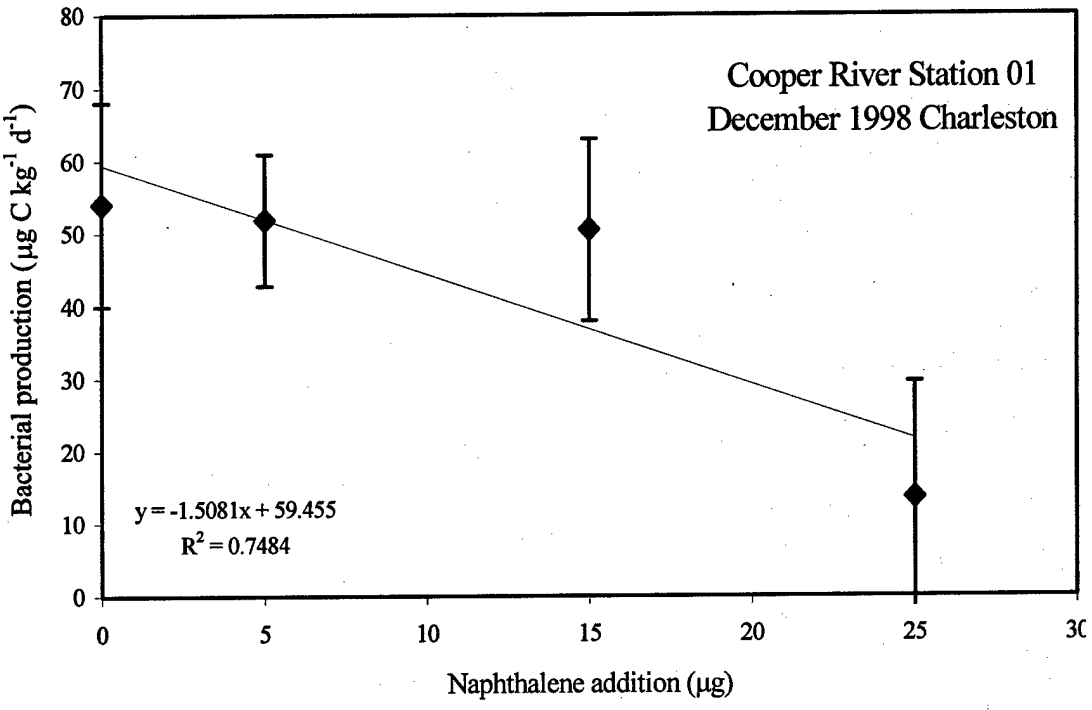

Fig. 30 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 01 during December 1998

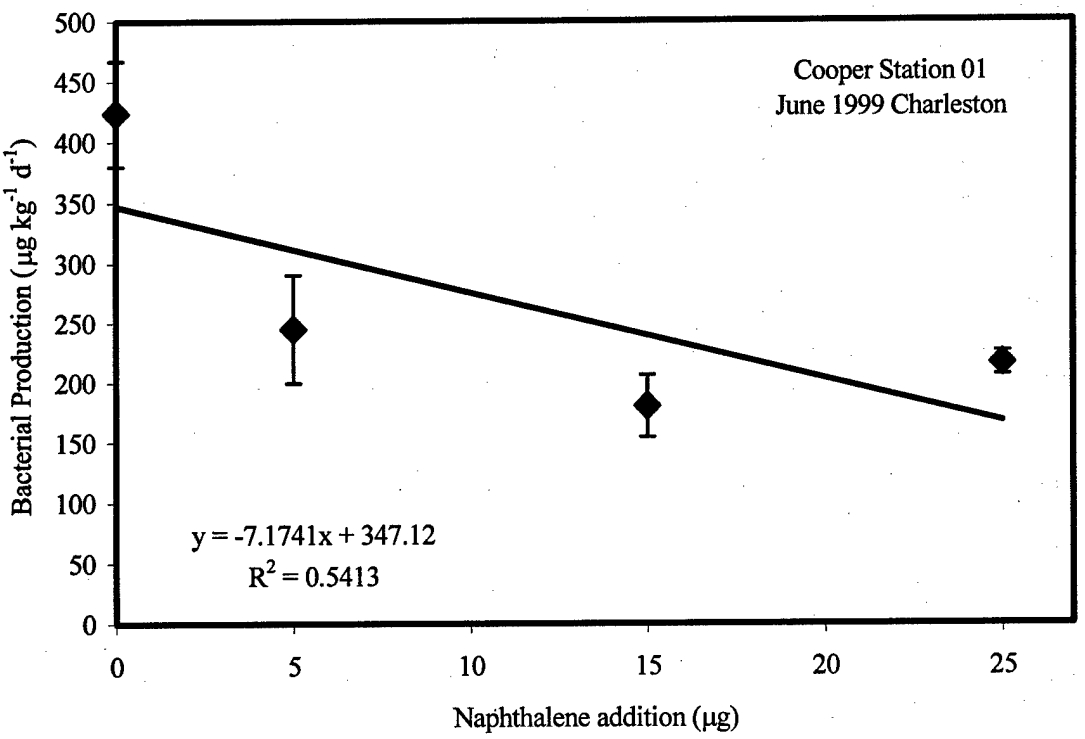

Fig. 31 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 01 during June 1999

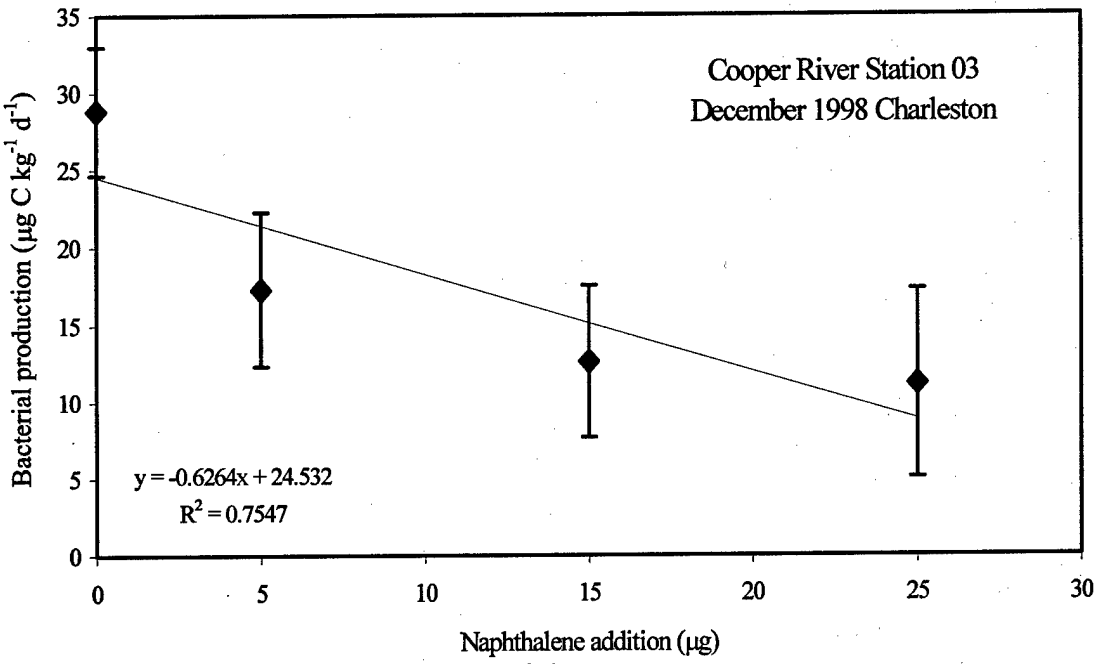

Fig. 32 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 03 during December 1998

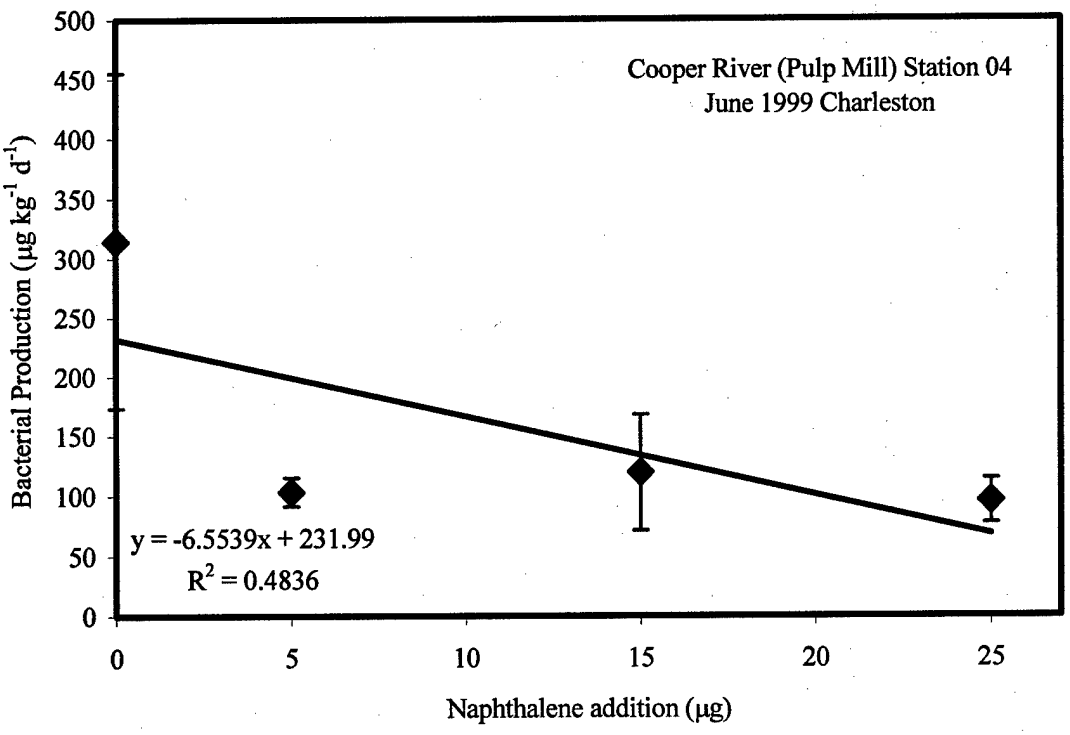

Fig. 33 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 04 during June 1999

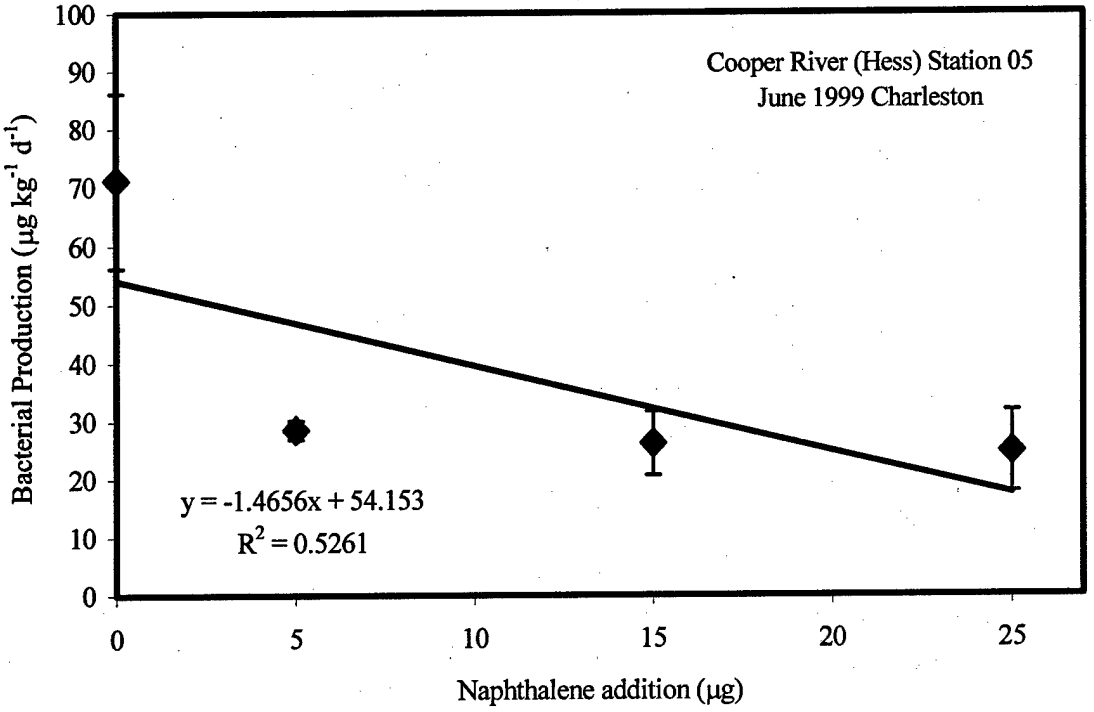

Fig. 34 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 05 during June 1999

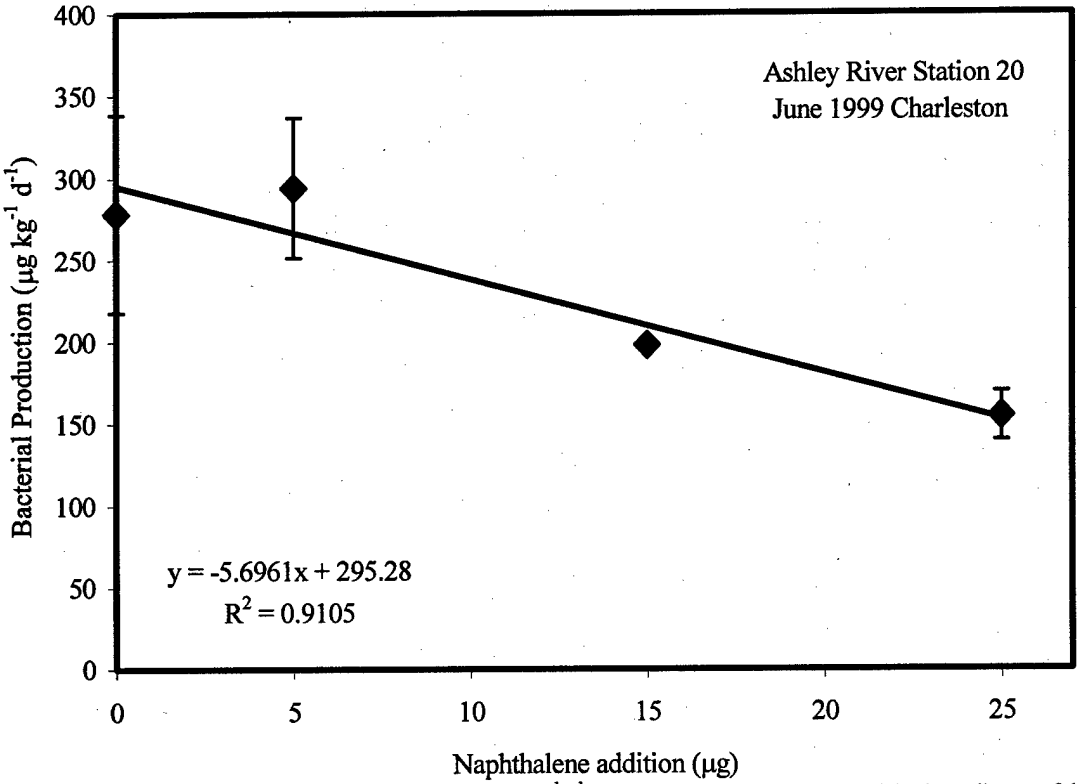

Fig. 35 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Ashley River station 20 during June 1999

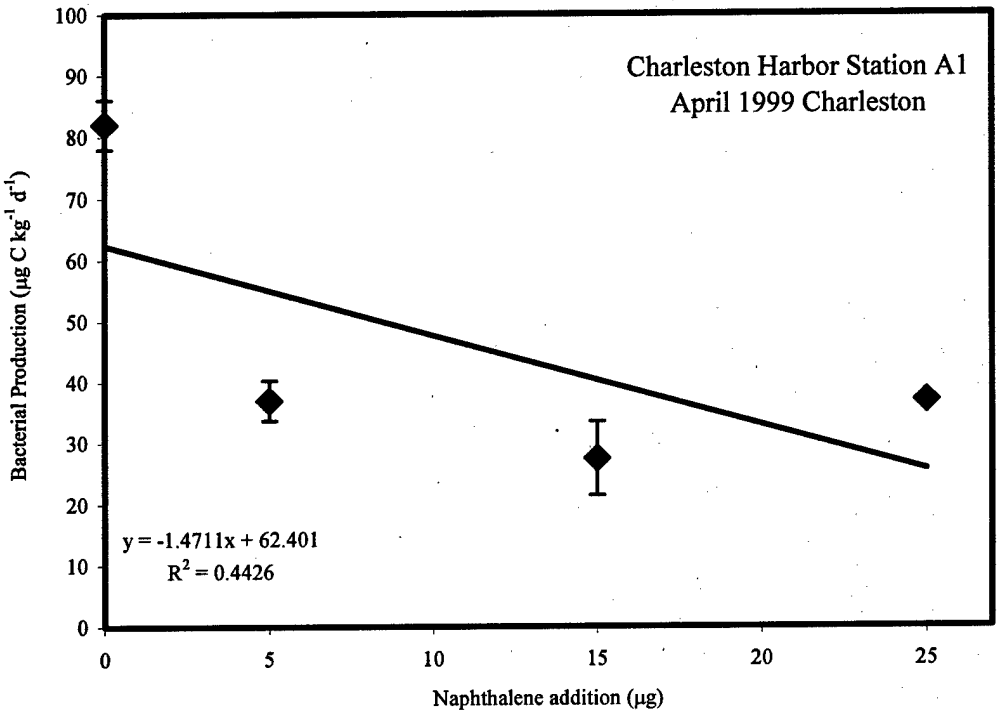

Fig. 36 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Charleston Harbor station A1 during April 1999

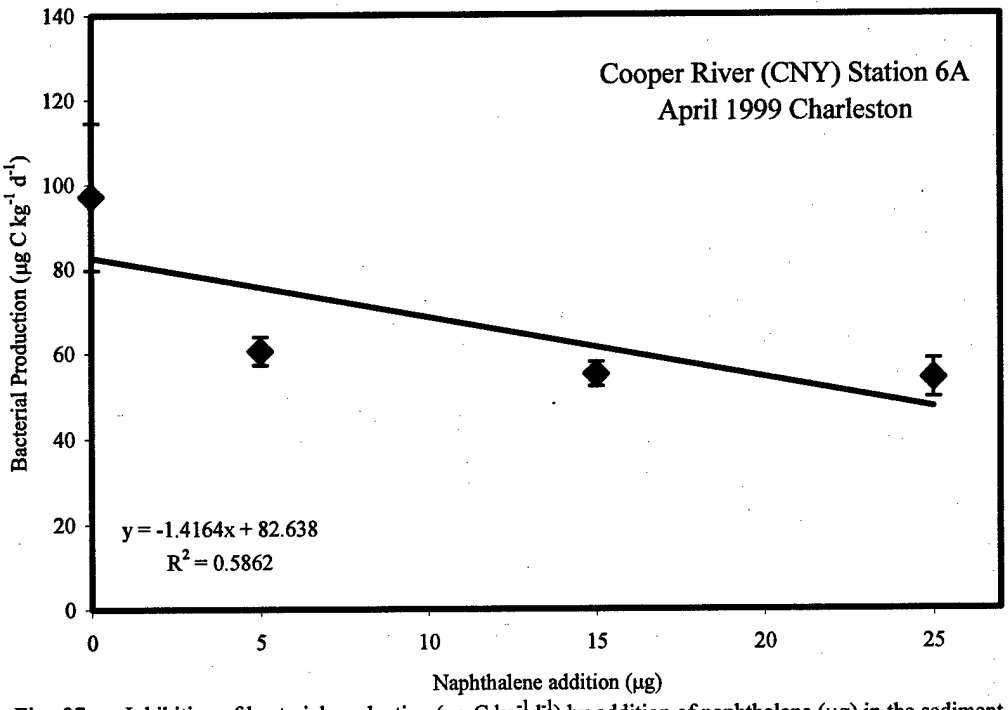

Fig. 37 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 6A during April 1999

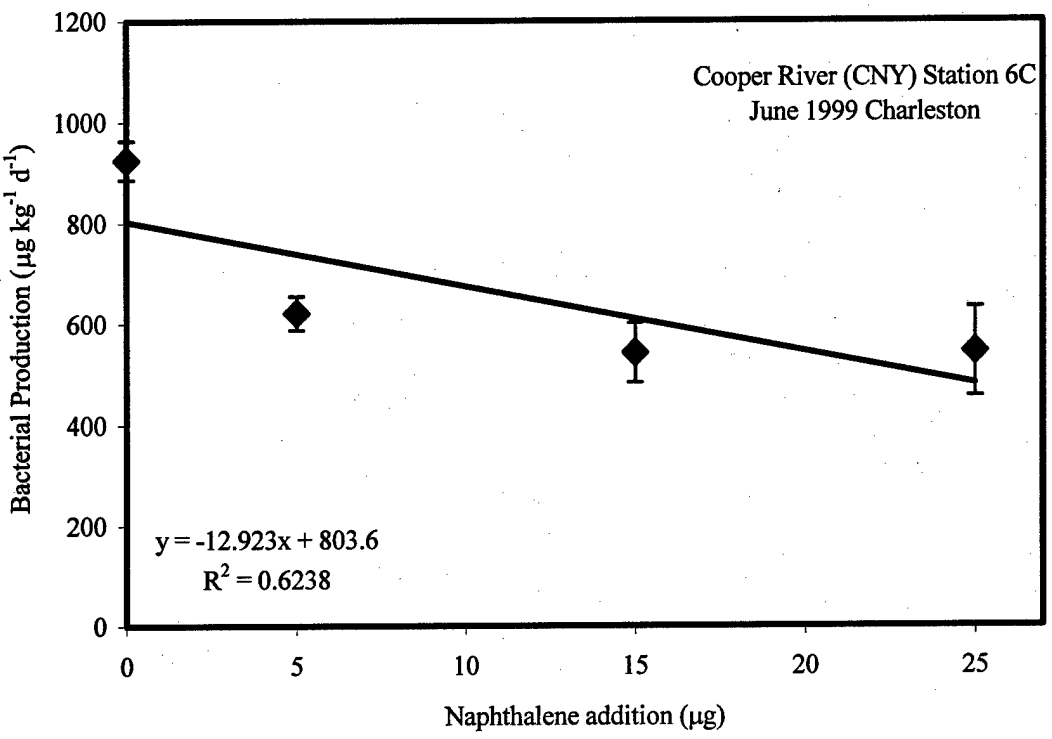

Fig. 38 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 6C during June 1999

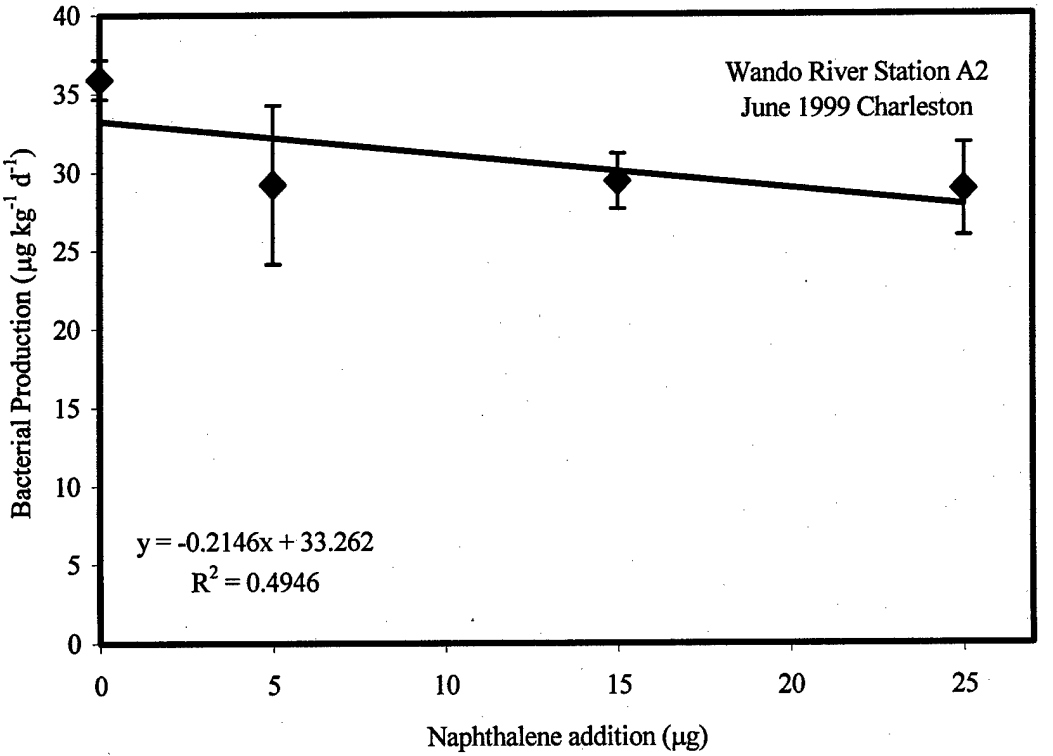

Fig. 39 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Wando River station A2 during June 1999

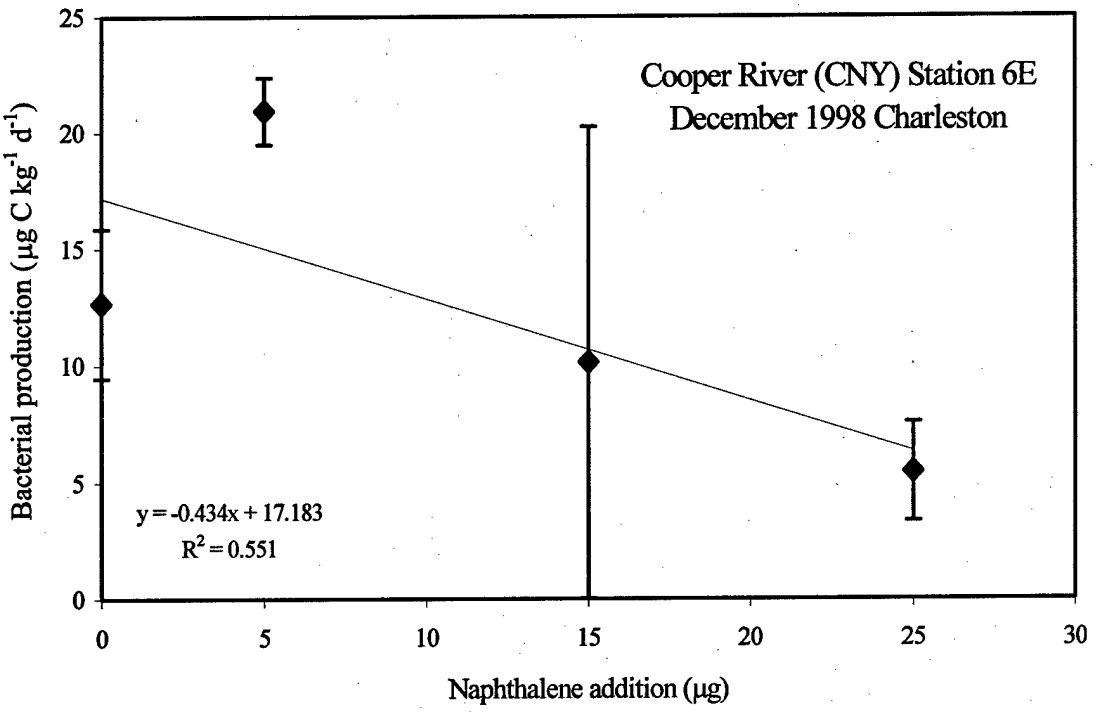

Fig. 40 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 6E during December 1998

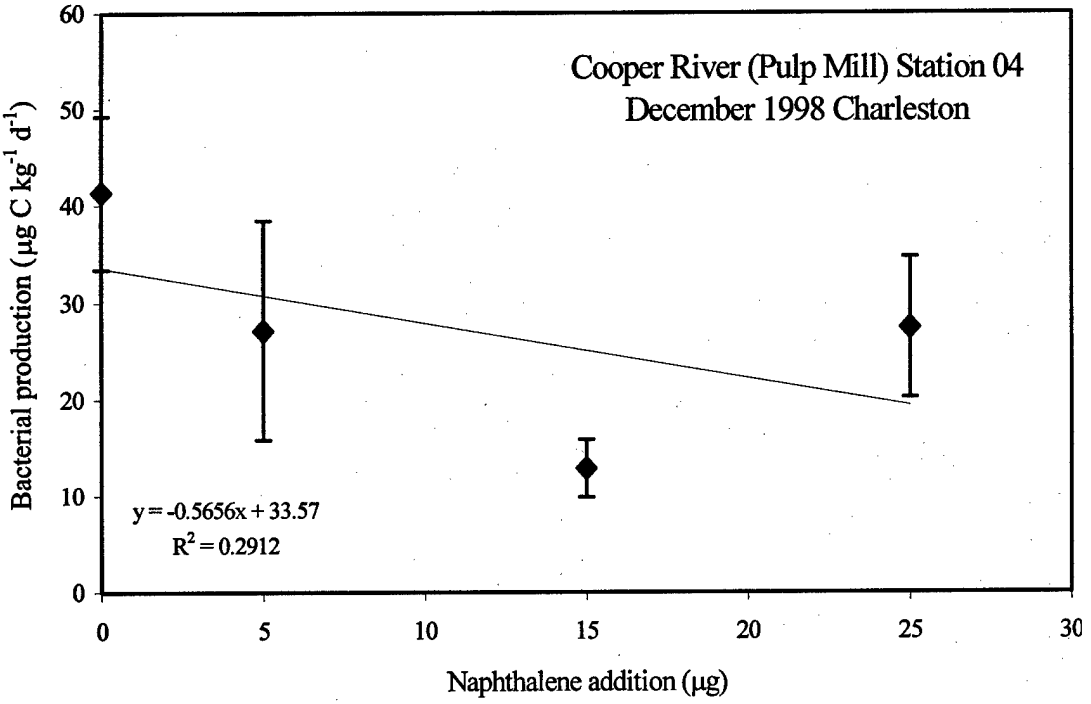

Fig. 41 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 04 during December 1998

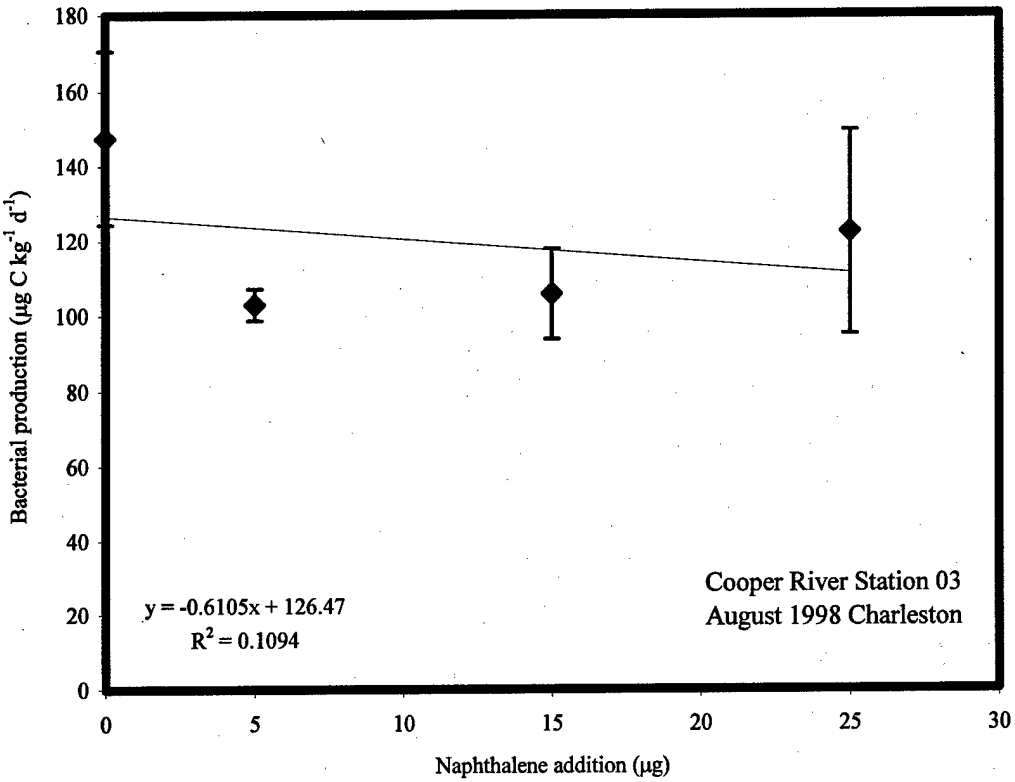

Fig. 42 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the Cooper River station 03 during August 1998

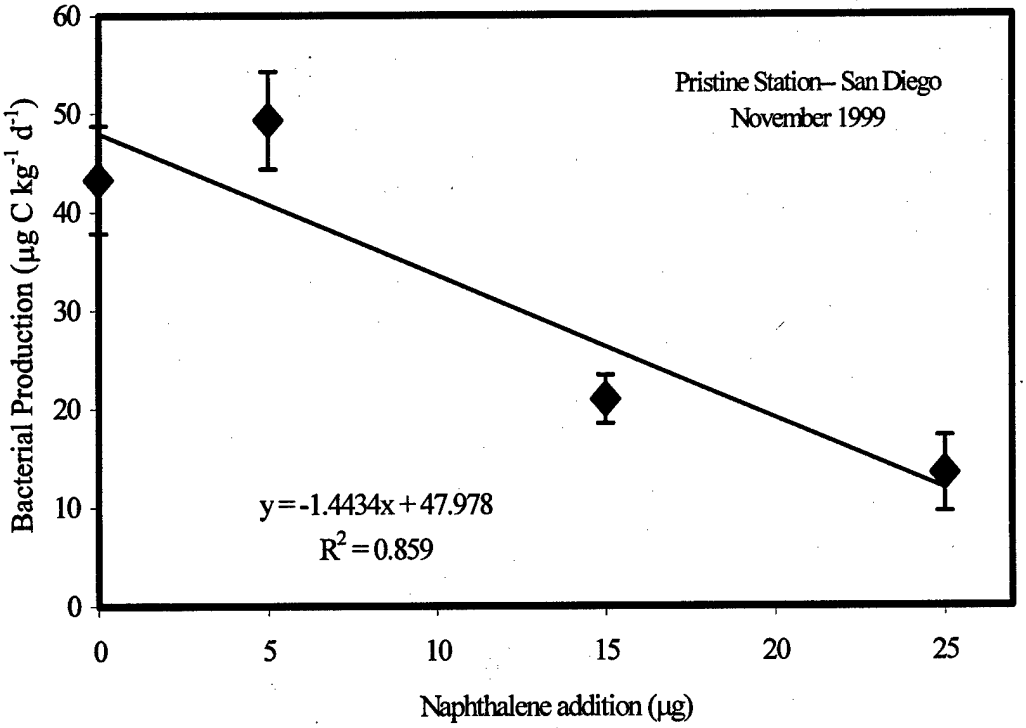

Fig. 43 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station Pristine during November 1999

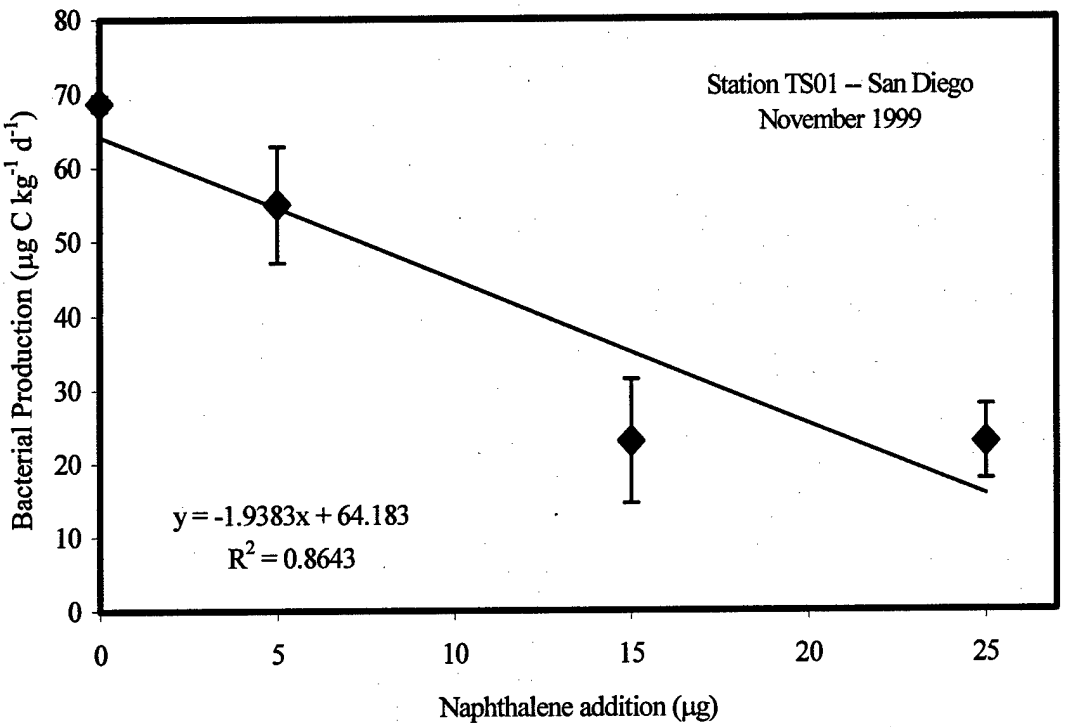

Fig. 44 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station TS01 during November 1999

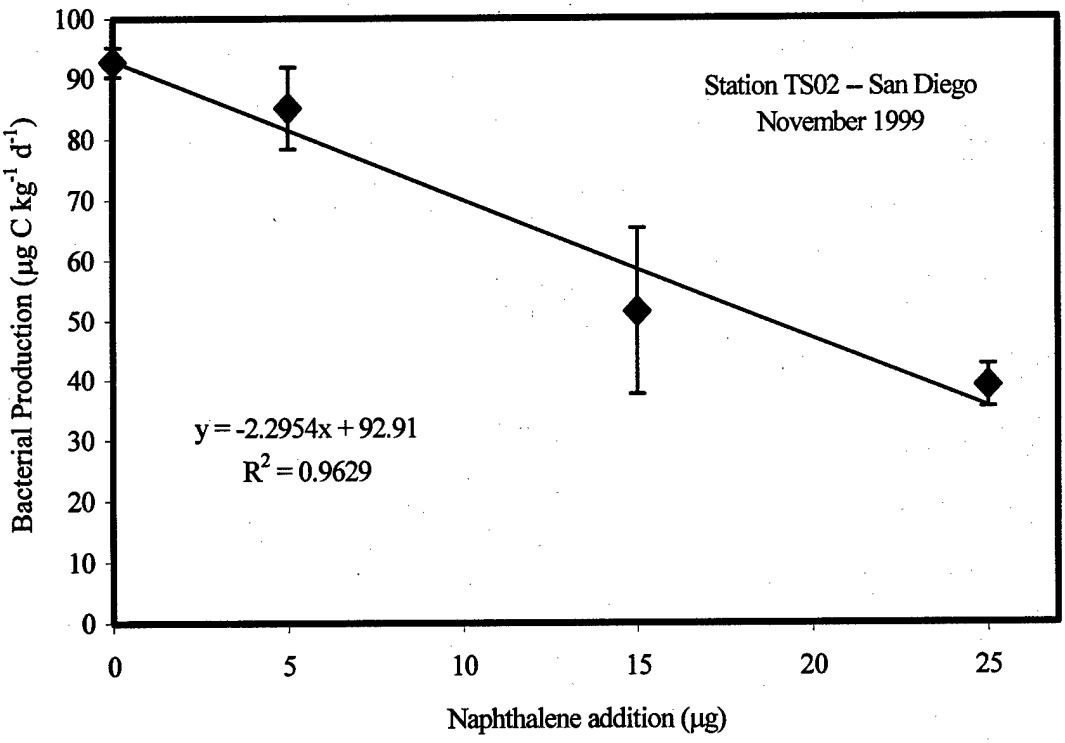

Fig. 45 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station TS02 during November 1999

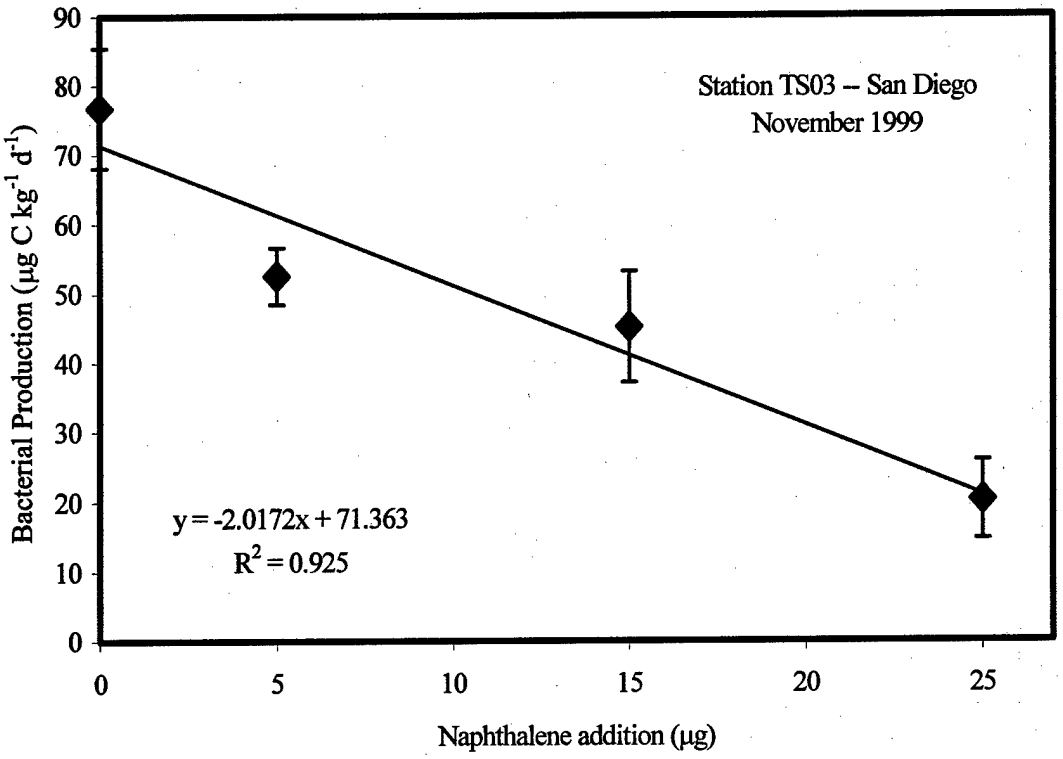

Fig. 46 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station TS03 during November 1999

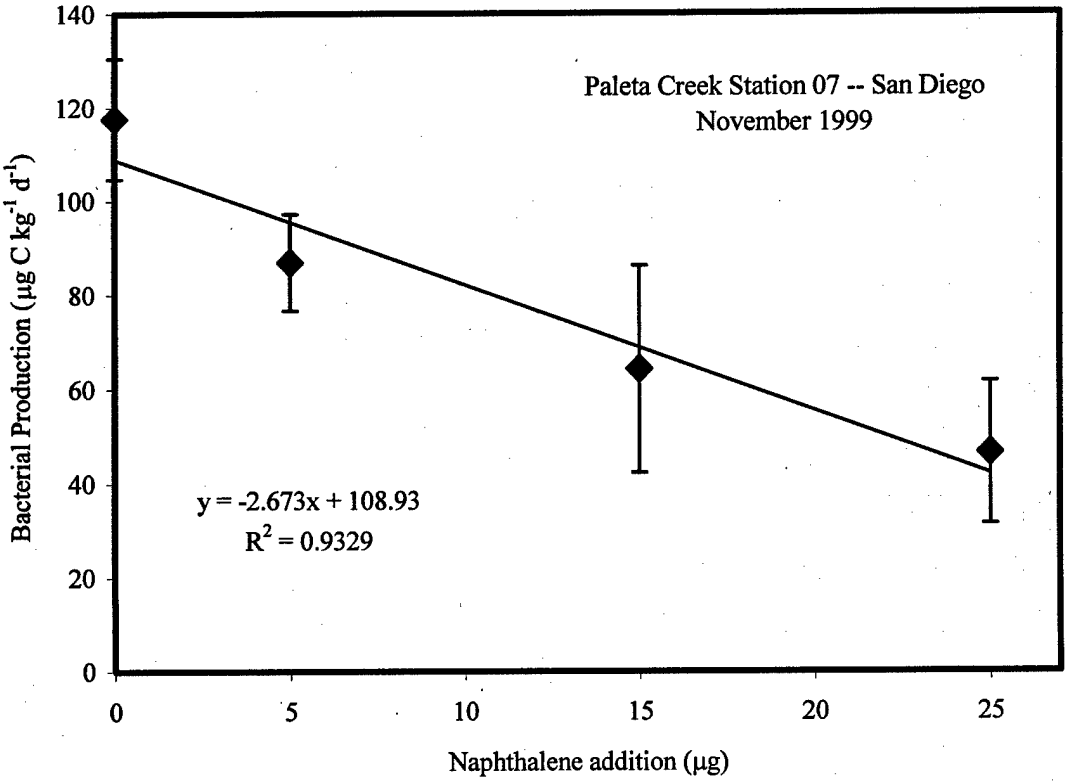

Fig. 47 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station PC07 during November 1999

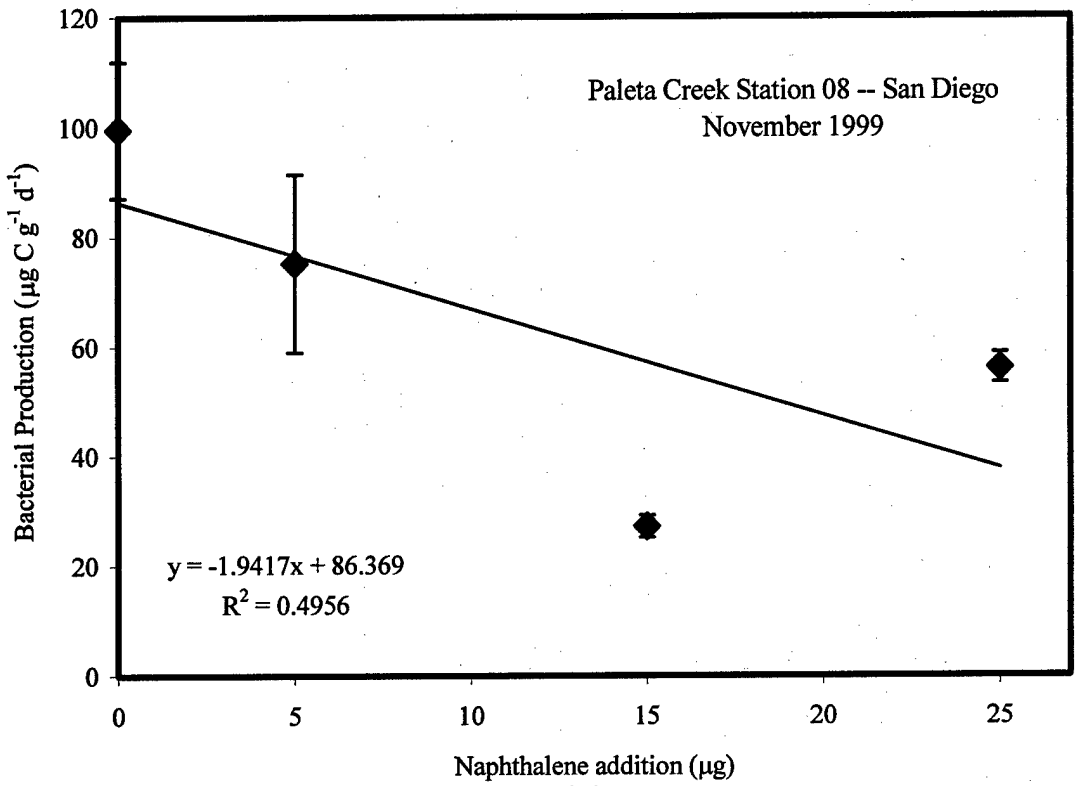

Fig. 48 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station PC08 during November 1999

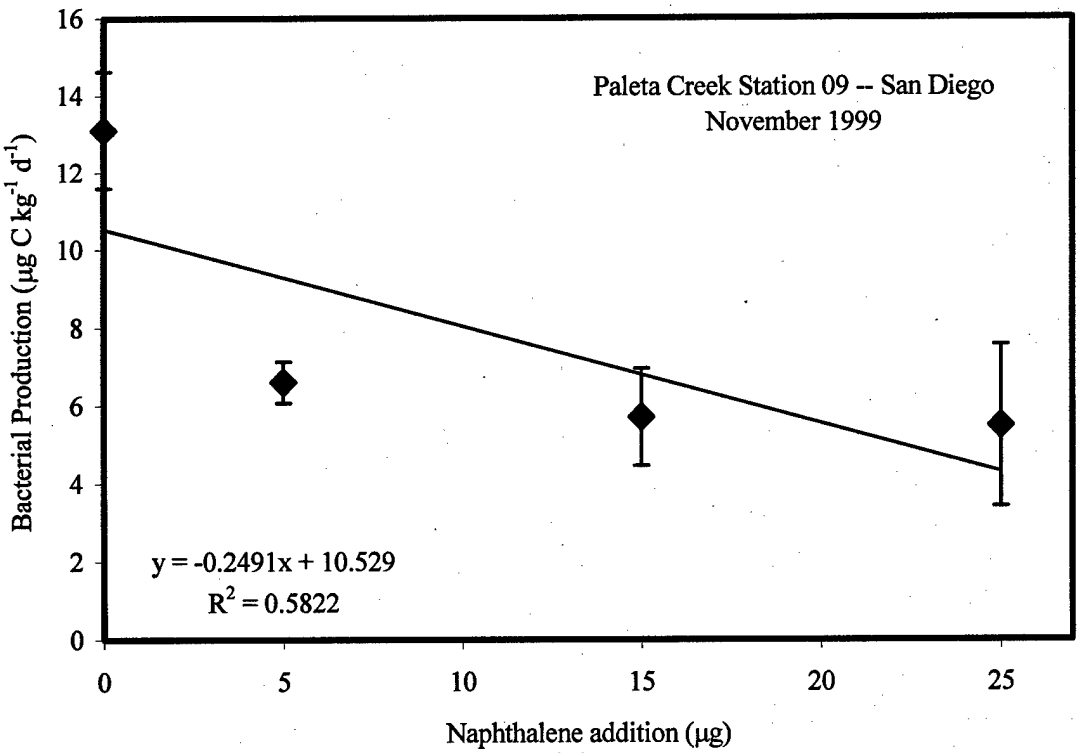

Fig. 49 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station PC09 during November 1999

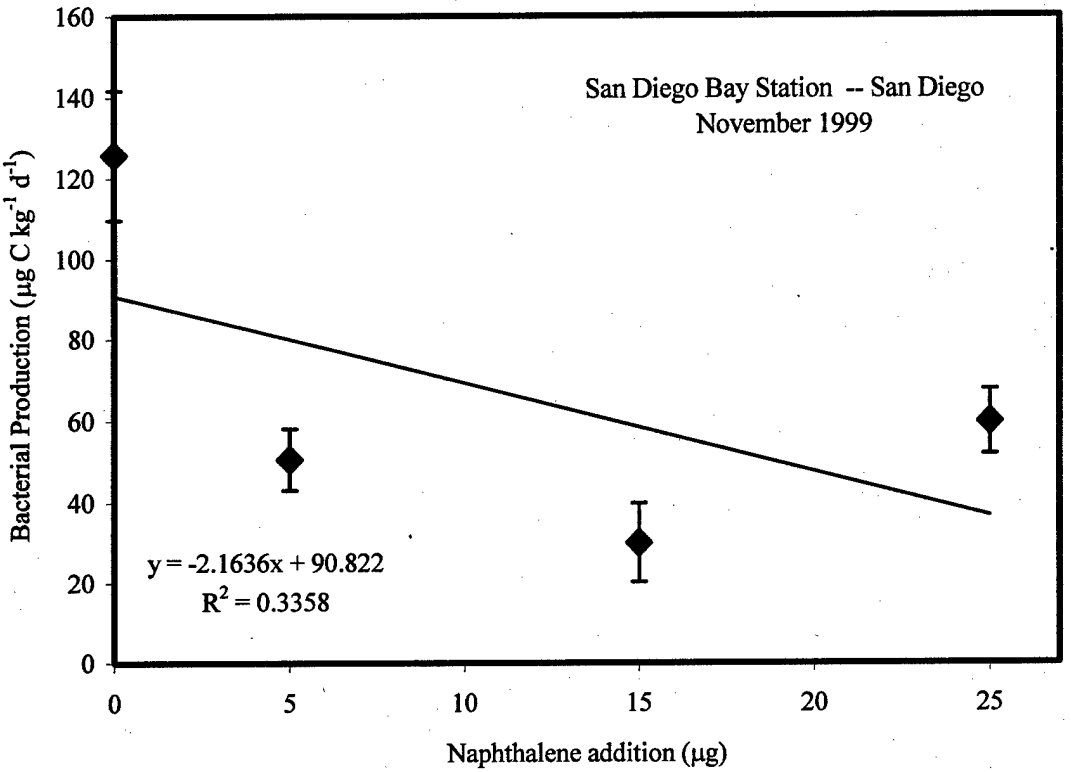

Fig. 50 — Inhibition of bacterial production (µg C $kg^{-1}d^{-1}$) by addition of naphthalene (µg) in the sediment of the San Diego station SDB during November 1999

Upper Delaware River System

During two research cruises in December 1998 and May 1999, the surface sediments were sampled in the Philadelphia Navy Yard Reserve Basin (RB04, -08, -10), and in the adjacent Schuylkill River (3, 6) and the Delaware River (9, 10, 12, 16). Possible sources of volatile organics to the surface sediments include historic petroleum inputs from ship activity in the Reserve Basin, effluent from petroleum refineries along the Schuylkill River (Pohlman et al. 2002), and an industrial outfall near Station 9 in the Delaware River.

Linear regressions described the inhibitory effect of naphthalene on bacterial production ($r^2 > 0.83$) at three stations including samplings at the Reserve Basin station RB04 (May: Fig. 51), one Schuylkill station 3 (December; Fig. 52) and one Delaware River station 12 (May; Fig. 53). Naphthalene additions actually stimulated production ($r^2 = 0.809$) in the sediment nearest the industrial outfall in the Delaware River at station 9 (December; Fig. 54). Naphthalene addition appeared to have little effect on production at three samplings: Schuylkill station 3 (May; Fig. 55), Delaware River station 10 (December; Fig. 56) and Reserve Basin station RB10 (May; Fig. 57). Finally, naphthalene appeared to inhibit production but the effect was poorly described by the linear regression ($r^2 = 0.551$ to 0.651) in May samples from the Reserve Basin station RB08 (Fig. 58), the Schuylkill station 6 (Fig. 59), and the Delaware River stations 10 and 16 (Figs. 60 and 61).

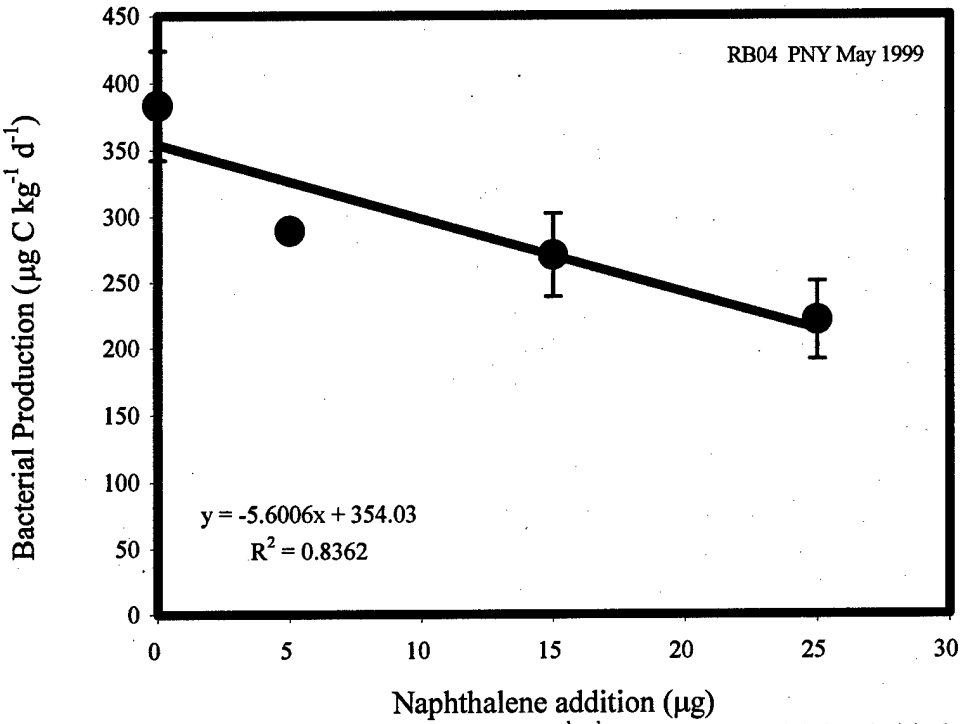

Fig. 51 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Philadelphia Reserve Basin station 04 during May 1999

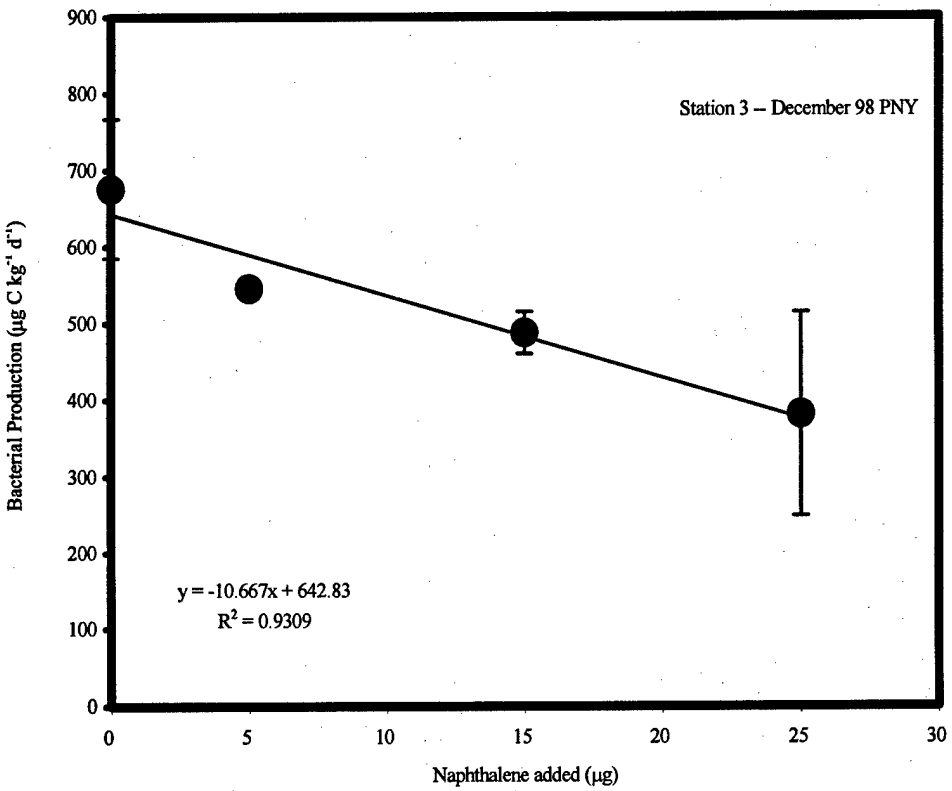

Fig. 52 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Delaware River station 03 during December 1998

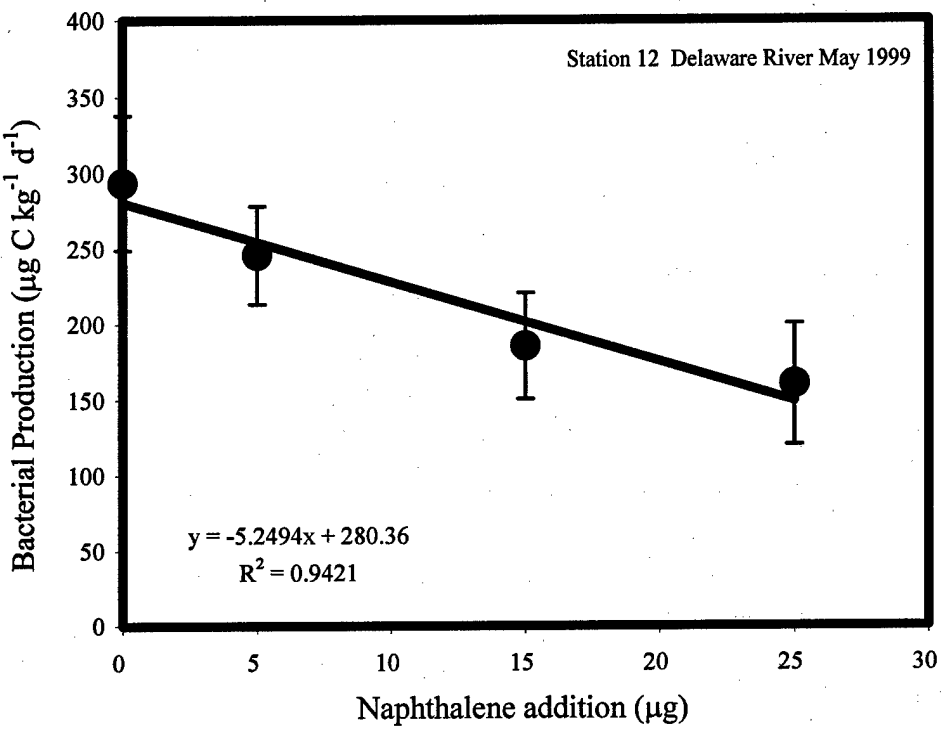

Fig. 53 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Delaware River station 12 during May 1999

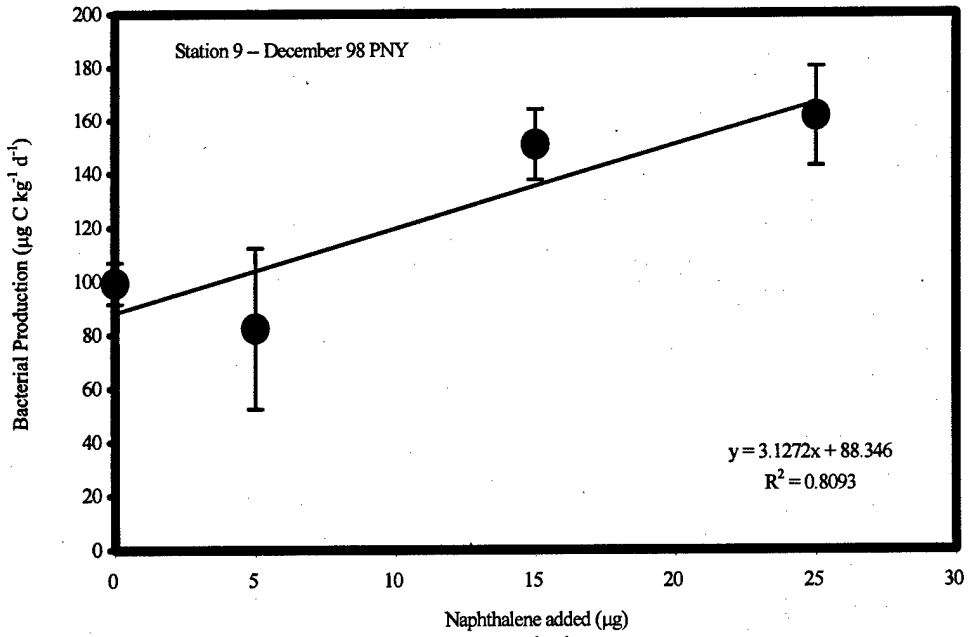

Fig. 54 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Delaware River station 09 during December 1998

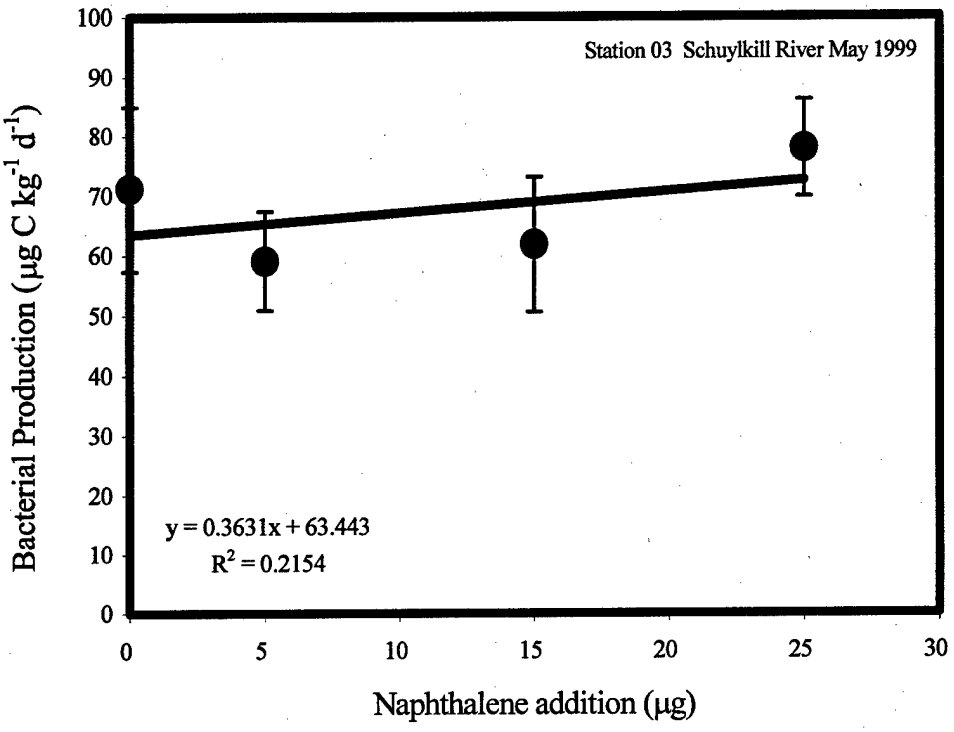

Fig. 55 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Schuylkill River station 03 during May 1999

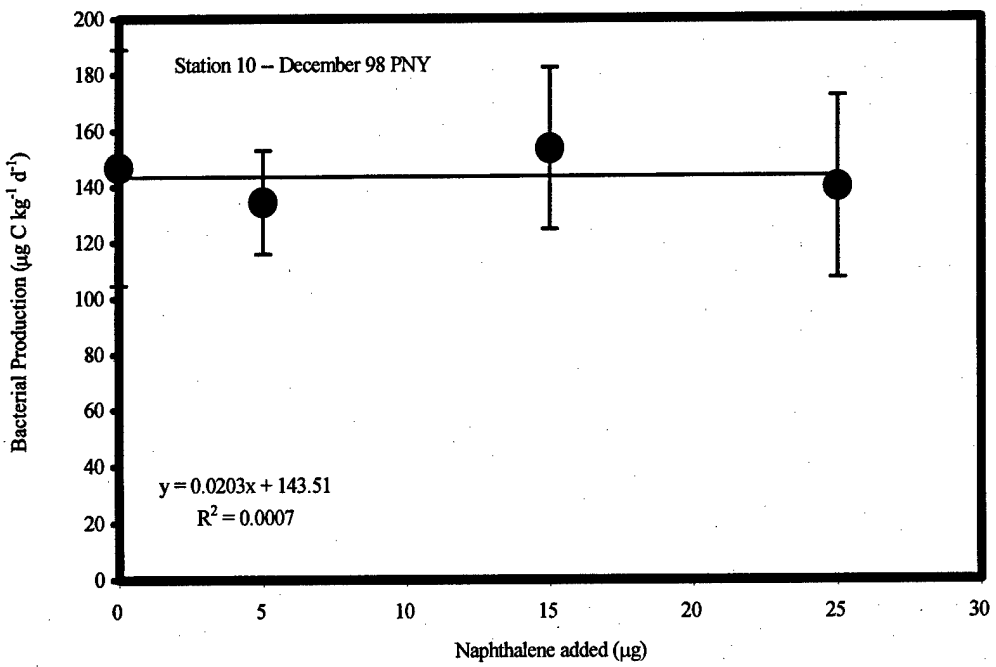

Fig. 56 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Delaware River station 10 during December 1998

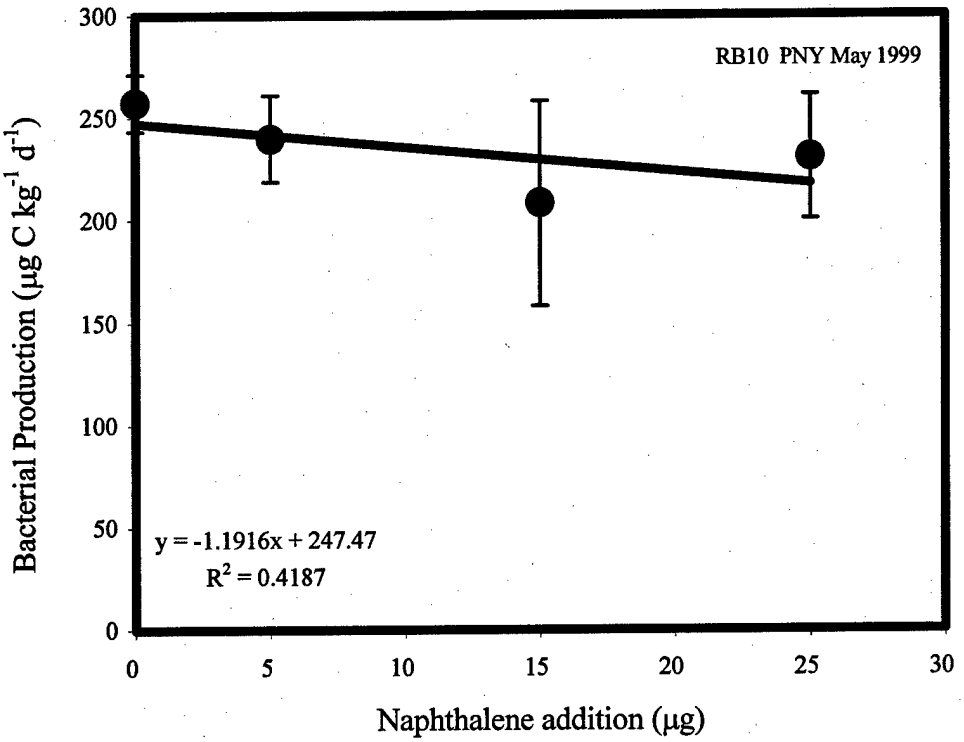

Fig. 57 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Philadelphia Reserve Basin station 10 during May 1999

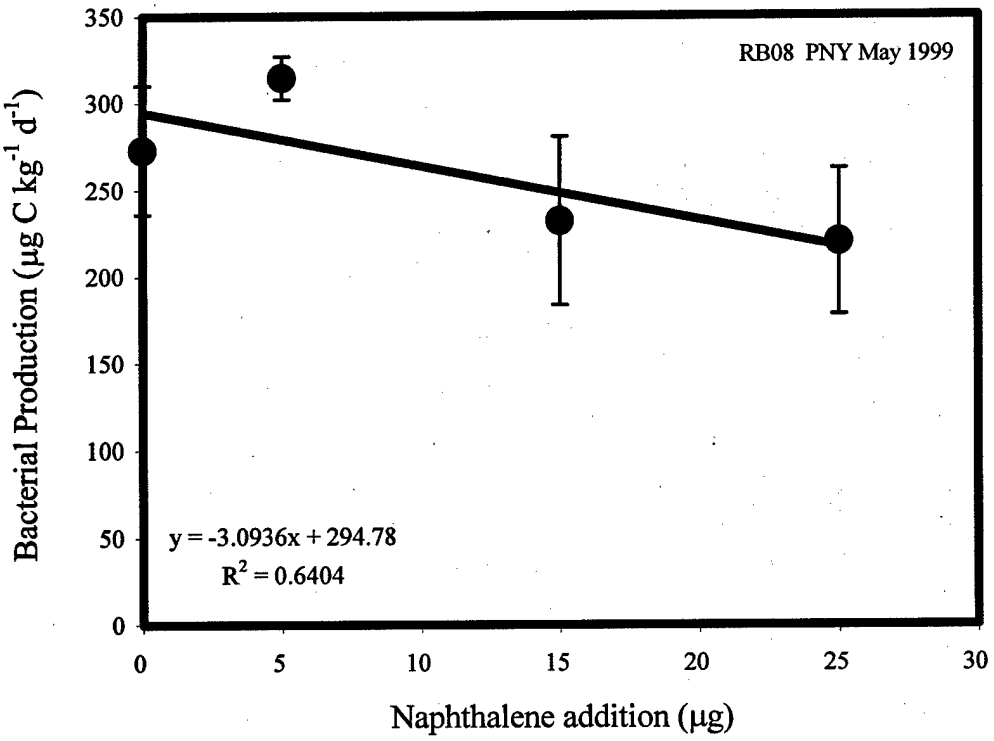

Fig. 58 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Philadelphia Reserve Basin station 08 during May 1999

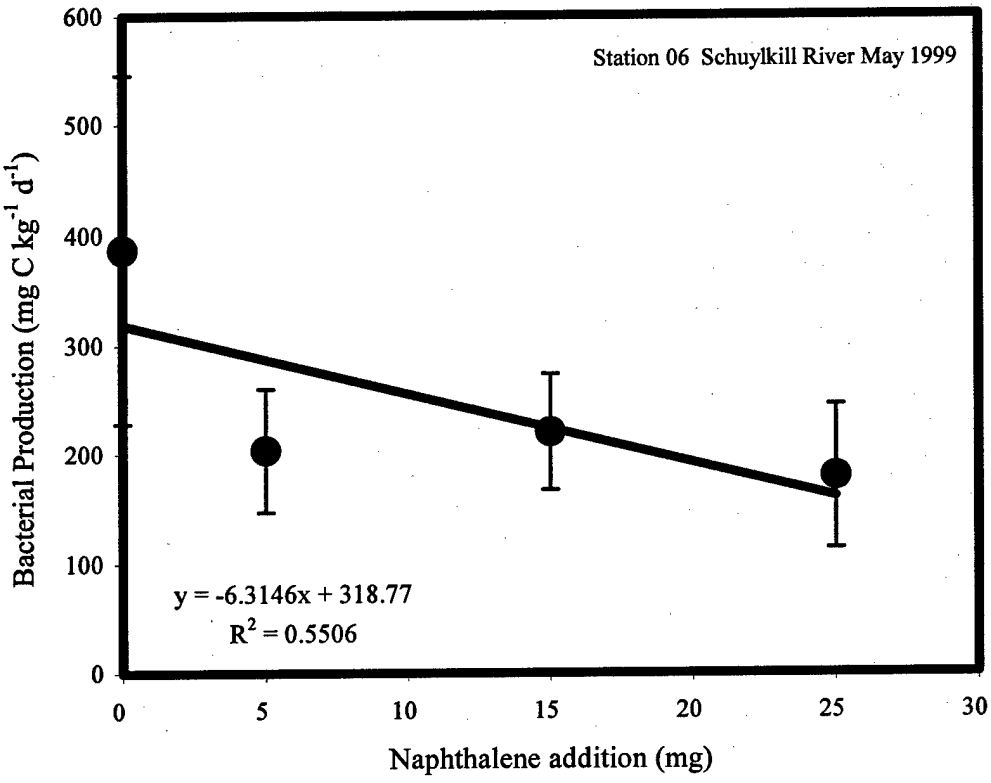

Fig. 59 — Inhibition of bacterial production (μg C kg^{-1} d^{-1}) by addition of naphthalene (μg) in the sediment of the Schuylkill River station 06 during May 1999

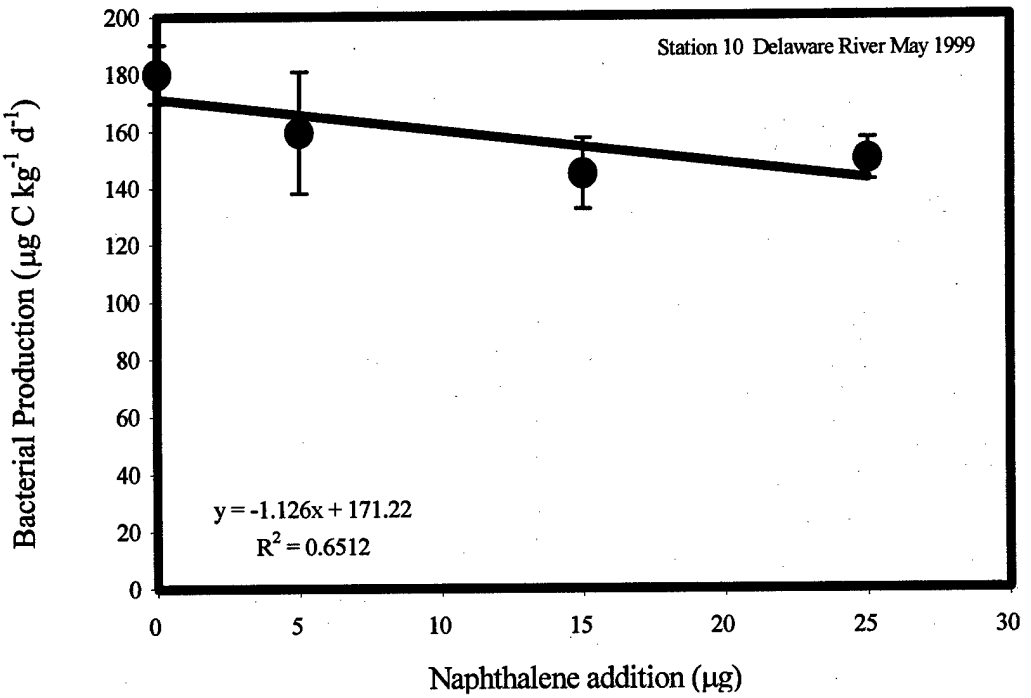

Fig. 60 — Inhibition of bacterial production (μg C kg^{-1} d^{-1}) by addition of naphthalene (μg) in the sediment of the Delaware River station 10 during May 1999

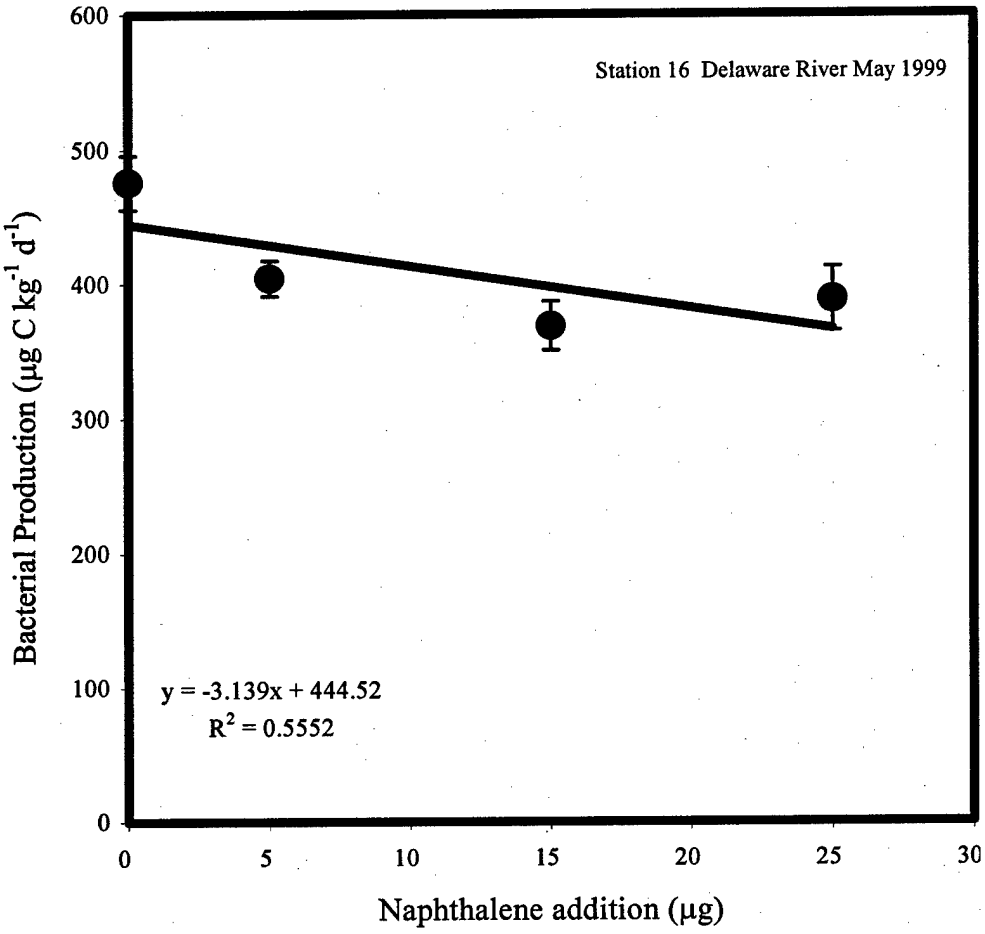

Fig. 61 — Inhibition of bacterial production (µg C kg^{-1} d^{-1}) by addition of naphthalene (µg) in the sediment of the Delaware River station 16 during May 1999

In most samples from these three ecosystems, leucine incorporation rate was reduced by the addition of naphthalene to the assay, as would be expected if most bacterial assemblages were not adapted for chronic exposure to volatile organics. Naphthalene can inhibit bacterial metabolism even if the assemblage has been exposed to low concentrations (10 µg L^{-1}) for a relatively long time (30 days) (see review by Capone and Bauer 1992, Bauer and Capone 1985). In stations from two sites nearest to known industrial outfalls, Delaware River station 9 and Cooper River station 4, naphthalene addition actually increased the rate of bacterial production in the surface sediment.

DISCUSSION

Bacterial production was measured in the sediments and surface waters of the watersheds adjacent to three Navy properties in Charleston, Philadelphia, and San Diego. Replicate samples were treated with naphthalene as a measure of the organotolerance of the natural bacterial assemblage. Samples from most stations exhibited some decrease in production with increase in the amount of naphthalene added to the heterotrophic production assay. At a couple of stations, naphthalene stimulated bacterial production, though this only happened with two of the 64 measurements. The two stations were adjacent to known

outfalls of volatile organics: Charleston station 4 near a paper mill outfall, and Philadelphia station 9 near another industrial outfall.

While several stations were always inhibited by naphthalene, there was variability between sampling times. This suggests the importance of multiple samplings to address both seasonal variations in the microbial assemblage, as well as the episodic nature of toxic releases from industrial operations. Many stations near the two cited outfalls had complex responses. For instance, stations upriver of the CNY in the Cooper River were often inhibited to some extent by low naphthalene additions (5 μg) but had little additional reduction at higher naphthalene additions (15 and 25 μg). One explanation is that a relatively high proportion of the bacterial assemblage is organotolerant, or more precisely, a large proportion of the cells contributing to heterotrophic production is organotolerant. The low concentration of naphthalene may have inhibited the relatively small proportion of organosensitive strains. Hudak et al. (1988) found that both naphthalene and phenanthrene acutely inhibited bacterial production (using thymidine incorporation) but stimulated production in longer-term exposures (more than 12 h). It is possible that the proportion of organosensitive strains correlates with the length of time since the last exposure to high VOC concentrations but this was not measured in this study. The likely source of elevated VOC input to this area of the Cooper River is the paper mill, which has an outfall at Station 4 permitted to discharge 20 million gallons per day of organic rich effluent (Van Dolah et al. 1990). Van Dolah et al. (1990) report that "this is the largest source of organic wastewater in the Cooper River and could dominate organic matter concentrations in the areas adjacent to the discharge. However, the effect was only apparent in the bottom waters over an 8 to10 RK distance downstream from the paper mill." As point of reference, the CNY sediments are located 2 to 9 km downstream of the paper mill.

Although we used naphthalene to represent exposure to other VOCs, the response of the assemblage might be similar if other organics were used in the assay. The general cellular response to VOCs, which is to alter the cell membrane structure, would not be expected to be specific to naphthalene addition but rather exposure to any high VOC concentration (Beney and Gervais 2001). There are other environmental stresses, such as high temperature or salinity change, which could promote a similar alteration in cell membrane structure that would confer organotolerance without prior exposure to VOCs. We compared temperature and salinity across sampling stations and could find no evidence that changes in assemblage response were the result of such environmental variation (data not shown). In general, the water temperature across a site differed between sampling events but was very similar between all stations for a given survey. The salinity at both San Diego and Philadelphia was also very similar across each respective site, with San Diego being marine water and Philadelphia being freshwater. Salinity between Charleston stations was different but there were no trends when salinity was compared with response to naphthalene (data not shown).

The response of bacteria to increase their organotolerance involves specific and well-documented changes to the cell membranes and can involve both short- and long-term responses. The short-term responses are primarily to maintain the cell's viability when exposed to an abrupt change in environmental condition. These responses include *cis* to *trans* isomerization of unsaturated fatty acids in the bacterial membrane (Loffeld and Keweloh 1996). This conversion results from the direct isomerization of the double bonds in the *cis* fatty acid and increases the membrane viscosity, which prevents solvent penetration (Morita et al. 1993). In addition to this rapid response, a long-term response to the presence of volatile organics involves changes in composition of the polar head groups in the bacterial membranes (Beney and Gervais 2001). The phosphatidylethanolamine concentration in the cell membrane decreases resulting in increased phospholipids cohesion, which affords the strain an additional protection from high nonpolar solvent concentrations (Beney and Gervais 2001). Because of the high correlation between membrane fluidity and cell resistance to stress, fluidity values have been used as

measure of organotolerance (Beney and Gervais 2001, Beney et al. 2001, Giraud et al. 2000, Swan and Watson 1997).

Once the immediate threat of cell damage or lysis has been overcome, bacterial strains that can catabolize the volatile organic or associated carbon sources will be selected for over noncatabolizing components of the assemblage. Strains amongst the organotolerant assemblage that cannot degrade compounds like naphthalene can acquire the necessary cellular machinery from other strains through horizontal gene transfer. There is growing evidence that transfer of naphthalene catabolic genes on plasmids is an important means of natural selection and community adaptation in hydrocarbon-contaminated sites (Stuart-Keil et al. 1998). In fact, the presence of contaminants like coal-tar may play a role in increasing the rate of gene transfer in nature by increasing the amount of cell-to-cell contact amongst proliferating members of the assemblage (Ghiorse et al. 1995, Stuart-Keil et al. 1998).

Once the assemblage is exposed to high concentrations of naphthalene, organotolerant strains that can catabolize naphthalene can rapidly increase in abundance. Dramatic change in community structure took place using samples from the bottom boundary layer (nepheloid) collected from Philadelphia Station 9 (industrial outfall), where sediment production was stimulated by naphthalene. Within 72 h of exposure to naphthalene, the most abundant strains (based on DGGE genetic analyses) were known naphthalene degrading strains (Castle et al. 2003). The time scales for recovery of the assemblage back to organosensitive strains is more poorly understood but may be based on protist grazing rate on bacteria, selective pressure from other extreme environmental conditions like high organic matter concentrations or nutrient competition (Morrison and Alexander 1997).

Submerged sediments in estuarine ecosystems can be periodically exposed to VOCs through a variety of common mechanisms including release from industrial outfalls. Bacteria amongst the assemblage can be either damaged or can alter the fatty acid composition in their cell membrane to become organotolerant. Adapted members of the microbial community that can catabolize VOCs may be responsible for much of the metabolic activity of the assemblage. Further exposure to VOCs, such as naphthalene, would be expected to have little negative impact on the rate of metabolic activity of the adapted assemblage. Although we do not know the exact time scales involved from exposure to adaptation, in this survey, we were able to identify two sediment stations that consistently harbored bacterial assemblages that were organotolerant. This report represents one line of evidence that VOCs are being released at such a rate from these two industrial outfalls that the natural bacterial assemblage in adjacent sediments is frequently impacted.

Although microorganisms are not considered a receptor in estuarine ecosystems, this assay provides an understanding of microbial community structure that could help assess sublethal effects in contaminated sediments and act as an early-warning system for effects on higher organisms (Eismann and Montuelle 1999). Some researchers have gone so far as to suggest that microbial ecotoxicity be incorporated into Environmental Risk Assessments (Babich and Stotzky 1985). In any event, such information should raise awareness that these areas may be a risk to the health of higher organisms and may impact adjacent ecosystems. Additional data on contaminant transport and biodegradation are currently being collected to help understand the consequences of these inputs to the adjacent watershed or downriver sediments.

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Appendix

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Boyd, T.J., M.T. Montgomery, B.J. Spargo, R.B. Coffin, J.K. Steele, J.P. Pohlman, and D. Velinsky. Characterization of Intrinsic Bioremediation within the Philadelphia Naval Complex Reserve Basin. NRL Technical Report, NRL/PU/6115--99-374 (1999).

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